THE EFFECT OF VARIED VITAMIN B INGESTION UPON THE APPETITE OF CHILDREN

FREDERIC W. SCHLUTZ AND ELIZABETH M. KNOTT
Department of Pediatrics, The University of Chicago
WITH THE COOPERATION OF NERINNE ISAACSON STAGE
Country Home for Convalescent Crippled Children
AND
MARTIN L. REYMERT
Mooseheart Laboratory for Child Research

TWO FIGURES

(Received for publication November 13, 1937)

A recent editorial in the Journal of the American Medical Association ('37) calls attention to the point that additional data are necessary in order to determine the optimum vitamin B requirement of children.

Studies by Gaynor and Dennett ('34), Morgan and Barry ('30), Summerfeldt ('32), Ross and Summerfeldt ('35) and Poole, Hamil, Cooley and Macy ('37) would seem to indicate that an increase in vitamin B ingestion is frequently beneficial to infants and children. Knott ('36) reported data to show that preschool children retained increasingly greater amounts of vitamin B as the level of vitamin B in their diets was increased. In commenting on Knott's results, Sherman and Sherman ('37) question whether higher intakes of vitamin B are optimal because of the danger of 'forcing' growth.

Since the conclusions of Gaynor and Dennett were based chiefly on clinical evidence, and since Morgan and Barry, and Ross and Summerfeldt used only gain in weight as a criterion, the present study was undertaken to show the quantitative effects upon appetite of different levels of vitamin B intake when continued over a period of time.

THE JOURNAL OF NUTRITION, VOL. 15, NO. 5
MAY, 1938

EXPERIMENTAL PROCEDURE

Subjects. Two groups of children have been studied. The first group consisted of thirty-two children in the Country Home for Convalescent Crippled Children. The majority of these children were convalescing from either tuberculous bone lesions or osteomyelitis, but at the time of this investigation their physical condition appeared to be fairly normal. Only those children were selected for the study who were able to live a normal child's life, including school and out-of-door play.

To contrast with this first group, a second group of twenty-two normal girls were selected from the Mooseheart Home for Child Training. These children were living together in one cottage, under the supervision of a matron and cook who assumed full responsibility for the care of the girls.

The physical status of both groups of children is itemized in table 1. Theoretical weights for most of the children were calculated by means of [1] formulae from the Iowa Child Welfare Research Station. At the Country Home, sex, age, height and chest circumference (the latter being corrected for subcutaneous fat) were used to determine the theoretical weight. At Mooseheart, hip and knee widths were also used in the formula for the theoretical weight. The deviation of the average weight for each child from his theoretical weight is given in table 1.

Diets. At both the Country Home and at Mooseheart particularly well planned dietaries are employed. The children receive one serving of meat, at least three cups of milk, and two servings each of fruit and vegetables besides potatoes, in addition to simple desserts and refined and whole-grain cereals and bread each day. Raw fruits and vegetables are included several times a week, and eggs are given every other day. Throughout the study the above dietary was not changed at either institution, and supplements of vitamin B were made in addition to the regularly planned menus.

[1] Personal communication.

TABLE 1

Physical status of the children

GROUP I						GROUP II						
Child	Age	Sex	Average height	Weight		Child	Age	Average height	Weight		Time at the Home	
				Ave.	Dev.[1]				Ave.	Dev.[2]		
	years		*cm.*	*kg.*	*kg.*		*years*	*cm.*	*kg.*	*kg.*	*years*	*mos.*
C.J.	5	F	93.8	15.7	+0.5	G.S.	9	122.2	23.7	−0.9	0	4
J.A.	6	F	108.	16.8	−1.7	E.F.	10	131.1	24.5	−0.4	7	3
R.J.	6	M	101.4	17.1	+1.0	B.P.	10	136.1	26.2	−0.4	8	6
L.T.	5	F	108.5	17.3	−1.3	C.C.	10	128.3	26.9	−0.1	1	0
E.B.	5	M	106.4	18.4	+0.3	B.W.	10	138.7	27.8	−2.2	7	1
J.M.	6	F	108.9	18.8	−1.8	D.W.	11	135.6	28.7	0.0	8	4
H.G.	6	F	109.3	19.7	−0.7	M.C.	10	128.5	29.2	−1.4	3	4
D.P.	6	M	111.1	20.3	−1.6	K.E.	10	136.1	29.4	−0.9	8	6
G.L.	5	M	111.1	20.7	−1.0	B.R.	11	140.7	30.8	−0.9	7	5
M.W.	10	F	119.3	21.	−1.7[3]	B.A.	10	140.0	31.7	−1.3	2	7
G.P.	10	M	122.4	21.6	−0.4	M.P.	11	133.6	31.7	+0.7	4	4
Ed.P.	7	M	116.6	21.8	+0.8	I.W.	11	135.1	32.	+0.2	0	1
A.O.	6	M	120.	22.1	−2.6	M.R.	11	137.9	32.1	−1.6	5	7
W.K.	8	M	118.1	22.2	+0.6	I.M.	11	146.3	33.1	−3.7	8	5
E.Ku	9	M	118.4	22.3	−0.3	F.D.	10	136.4	33.5	−1.6	8	10
M.R.	9	F	117.5	22.6	−0.2	P.W.	10	134.6	34.3	+0.3	6	9
G.W.	9	M	127.4	23.9	−1.4	M.D.	12	137.4	35.5	+1.2	0	7
J.T.	9	M	128.9	23.9	−3.3[3]	D.S.	11	141.0	37.1	−0.2	0	4
A.S.	7	F	116.6	24.1	+2.8[3]	A.G.	11	150.0	38.9	−0.8	6	9
M.L.	9	M	128.7	25.0	−0.6	S.B.	12	153.4	39.1	−1.3	0	3
J.S.	7	M	132.	25.1	−3.5[3]	A.P.	11	145.5	40.9	+2.1	6	3
L.S.	8	M	128.9	25.7	−2.7	E.G.	11	152.1	43.2	+2.1	6	9
T.C.	9	F	126.0	26.2	−0.6	J.B.	11	143.8	45.8	+6.4	0	3
T.L.	8	M	129.5	26.2	−2.5							
J.B.	10	M	134.5	26.7	−3.7[3]							
E.S.	9	M	130.5	27.2	−0.9[3]							
E.Ka	9	M	133.4	27.3	−2.3							
D.H.	9	M	130.4	27.7	−0.4[3]							
B.G.	9	M	130.2	30.5	+2.4[3]							
C.C.	11	F	136.	30.6	−4.0							
L.L.	10	M	136.3	30.7	−3.7							
D.M.	10	M	135.	33.	+0.2							

[1] Theoretical weight calculated on basis of sex, age, height, and chest (corrected for fat).

[2] Theoretical weight calculated on basis of sex, age, height only.

[3] Theoretical weight calculated on basis of sex, age, height and body form.

Organization. The children at the Country Home were divided into three groups. During the first period group 1 received at breakfast 15 gm. of a refined wheat cereal [2]; group 2 received 15 gm. of a whole wheat cereal; and group 3 received the whole wheat cereal fortified with 3.3 gm. of a wheat germ preparation [3] which had been stabilized by removal of fat and moisture. At the end of the first 8 weeks, and again at the end of the second period, the groups were rotated so that each group received each of the three different cereals. These three periods served as a control for the remainder of the investigation. During a fourth period the children were given free choice of as much breakfast cereal as they desired (the kind of cereal being changed from day to day), and received in addition a supplement of 1 teaspoonful of the stabilized wheat germ at each meal (making a total of 20 gm. of wheat germ daily). During the two final consecutive periods of 4 weeks each, each child received daily 455 micrograms of crystalline vitamin B.[4] The stabilized wheat germ was given at each meal in a small amount of milk. The crystalline vitamin B was put in solution and added to the milk once a day during the first 4 weeks, and three times a day during the second 4 weeks of the final period.

At Mooseheart the investigation was divided into four periods, during the first and third of which the children received no supplement, while stabilized wheat germ was given in the second period and crystalline vitamin B [5] during the fourth period. The first two periods were of 3 months duration each, the last two of 4 weeks each.

Methods. To determine food consumption of the children, sample servings of each food offered were weighed at each meal. Standard measuring devices were then used in serving

[2] The refined malted wheat cereal was supplied through the courtesy of the Campbell Cereal Company, Minneapolis, Minn.

[3] The stabilized wheat germ, VioBin, was furnished by the VioBin Corporation, Chicago, Ill.

[4] Natural crystalline vitamin B was kindly supplied by Merck and Company.

[5] Synthetic crystalline vitamin B was supplied in the form of 0.1 mg. tablets of 'Betaxin' through the courtesy of the Winthrop Chemical Company.

each child so that all portions served would correspond to the weighed samples. At the Country Home complete records were kept at every meal of the number of servings of each food consumed by each child. Once a week the records were summarized and the average grams of food consumed daily were calculated. In addition, calculations were made (by means of standard dietary tables) of the average daily caloric and protein ingestion of each child. At Mooseheart the food consumption records were kept for seven consecutive days at the end of each 4-week period.

To determine the physical progress of the children throughout the study, body weights were taken every 2 weeks and height measurements were made once a month.

DISCUSSION OF RESULTS

Country Home results for cereal and wheat germ periods. The average grams of food consumed per day for each child at the Country Home have been summarized for each period in table 2. Of the seventeen children studied on all of the first three cereal periods, only four (J.M., G.L., E.Ku., and M.R.) showed a progressive increase in food intake as the cereal was changed from refined wheat to whole wheat to whole wheat plus 3.3 gm. stabilized wheat germ. The average grams of food intake for the three groups of children, however, for each of these three cereal periods were as follows:

Refined wheat	1591 gm. of food per child per day
Whole wheat	1598 gm. of food per child per day
Whole wheat + wheat germ	1607 gm. of food per child per day

It would thus seem that although the difference is small, the type of cereal may have an influence upon the child's appetite. Because the difference is small, these three periods have been grouped together as a control for further studies with the Country Home children.

The benefit of added amounts of wheat germ is more clearly brought out from a study of the results of a fourth period. It will be noted that when the wheat germ intake was increased to 20 gm. per day, the food consumption of every child without exception was definitely increased. The average food

TABLE 2

Average grams of food consumed per day, by each child, during each period

Group I. Country Home

PERIOD DATE	1. 9/16–11/10			2. 11/11–1/5			3. 1/6–2/2			4. 3/24–4/20	5. 8/12–9/8	6. 9/9–10/6
	Control period									Wheat germ (20	Crystalline vitamin B	
	1[1]	2[2]	3[3]	1[1]	2[2]	3[3]	1[1]	2[2]	3[3]			
C.J.			1243		1339					1681	1573	1522
J.A.	1584					1499		1208				
R.J.	1436					1429		1449				
L.T.					1617		1348				1933	1840
E.B.		1535		1526					1270			
J.M.		1332		1320					1425	1646		
H.G.		1487		1421					1476	1782	1760	
D.P.	1526									1965		
G.L.			1466		1458		1450			1708		
M.W.											1499	1441
G.P.		1630		1604					1551	2207		
Ed.P.			1740		1534		1686					
A.O.										1976	2192	2055
W.K.	1455					1343		1335		1980		
E.Ku			1759		1679		1662				2113	2036
M.R.			1555		1497		1458			2003		
G.W.										2064	2384	2340
J.T.											2168	1637
A.S.											1525	1395
M.L.	1835					1900		1958		2226		
J.S.											2197	2200
L.S.	1676					1764		1810		1967	2262	2302
T.C.	1495					1560		1630		1978	1949	1837
T.L.			2121		1923		1926			2261	2535	2518
J.B.											1969	1989
E.S.											1544	1896
E.Ka		1829		1873					1992			
D.H.											2154	2366
B.G.											2109	2139
C.C.										2309	2241	2128
L.L.										2055		
D.M.		2085		1931					1877	2301		
Ave.	1572	1650	1638	1613	1578	1583	1588	1565	1599		1972	1949
	1599									2003	1961	

[1] Refined wheat.

[2] Whole wheat.

[3] Whole wheat + 3.3 gm. wheat germ.

intake for the entire group for this fourth period was 2003 gm. per day as compared to an average of 1599 gm. for the first three cereal periods.

TABLE 3

Average grams of food consumed per day by each child, during each period

Group II. Mooseheart

PERIOD DATE	1. 8/13–20	2. 9/9–15	3. 10/7–13	4. 11/4–11	5. 12/2–8	6. 12/30–1/5	7. 3/3–9	8. 3/31–4/6
DIET	No supplement			Stabilized wheat germ			Control	Crystalline vitamin B [1]
				18 gm./day				
B.A.	1679	1768	1874	2435	2248			2041
C.C.	1692	1863	1859	2002	1951		1731	2015
M.C.	1574	1812	1851	1973	1842	1973	2072	1903
M.D.	1757	1926	1963		1904	2036	1841	2097
F.D.	1755	1874	1874	2186	1993	2214	1774	2122
K.E.	1721	1881	1807	1987	1942	2198		2242
E.F.	1522	1535	1737	1814	1737		1642	1938
E.G.	1708	1889	1976	2408	2583		2112	2442
A.G.	1886		1976		2472	3143	2612	2558
I.M.	1671	1834	1845	2018	1896	2212	1870	2122
M.P.	1721	1941	1938	2283	2003	2015	2024	1981
A.P.	1622	1928	1757	2281	2160	2585	1870	2537
B.P.	1654	1843	1810					
I.W.				1724	1781	2002	1609	
M.R.	1807	1922	1921	2185	2138	2414	1882	2255
B.R.	1730	1838	1813	2033	1902	2187	1688	1979
B.W.	1600	1793	1816	2012	1940	2069	1909	1967
P.W.	1835	1715	1736	1988	1887	2078	1750	2125
D.W.	1730	1832	1853	1937	1791	1962	1798	1964
D.S.	1764	2017	1907	2278	2133	2636	1933	2083
G.S.	1566	1722	1752	1848	1805	1923	1767	1929
J.B.		1826	1802	1851	1883	2238	1892	2068
S.B.		1645	1704	1881	1804	2077	1570	
Ave.	1700	1829	1844	2056	1991	2220		
	1791			2089			1873	2116

[1] Four 0.1 mg. tablets 'Betaxin' per day.

Mooseheart results for control and wheat germ periods. The average grams of food consumed per day for the children at Mooseheart have been summarized in table 3. During the first 3 months the children consumed an average of 1791 gm. of food per day. During the following 3 months, while they

were receiving about 18 gm. of wheat germ daily, the average food consumption increased to 2089 gm. per day. During a subsequent control period the daily intake decreased to 1873 gm. The increase with these children was not quite so great as that observed with the Country Home children. The Mooseheart girls, however, were more normal subjects, and the greater increase at the Country Home might have been due to a previous retardation of these children before the convalescent period during which they were studied.

Results with crystalline vitamin B. In order to determine whether the vitamin B of the wheat germ may have been responsible for its influence upon the appetite, crystalline vitamin B in amounts comparable to the vitamin B content of the wheat germ was given to both groups of children. At the Country Home the average grams of food consumed per day during the two successive 4-week crystalline vitamin B periods were 1972 and 1949. Thus the stimulation of appetite with crystalline vitamin B gave results quite close in amount to the average intake of 2003 gm. which was taken by the children during the wheat germ period. At Mooseheart similar results were obtained. During the crystalline vitamin B period the children consumed an average of 2116 gm. of food, as compared to 1873 gm. during the 4-week control period immediately preceding the crystalline vitamin B period, and the averages of 2089 gm. for the wheat germ period and 1791 gm. for the first 3-month control period.

In order to determine the magnitude of change in vitamin B ingestion which can affect the child's response, the vitamin B contents of the diets and supplements have been determined by means of standardized biological assay technic as described by Schlutz and Knott ('36) with the improvement in technic as noted by Knott and Schlutz ('37). The vitamin B contents of the different cereals, the stabilized wheat germ, and aliquots of the total amount of food served on representative days are given in table 4. Since the quantity of second servings consumed increased the amount of food intake from 1.5 to 2 times above the original servings for which vitamin B values are

recorded in table 4, the children received from 260 to about 420 international standard units of vitamin B per day. A change from refined wheat to fortified whole wheat cereal increased the daily vitamin intake by 33 units or about 10% of the total daily intake. Apparently larger increases than this

TABLE 4

The vitamin B contents of diets and supplements in terms of international standard units of vitamin B

	ASSAY RESULTS (TOTAL DOSE/TOTAL GAIN)	FACTOR TO CONVERT TO I.S. UNITS	UNITS/GM.	UNITS/DAY
Cereals				
Refined wheat	1.57	1.49	0.43	6.5
Whole wheat	0.46	1.49	1.45	21.7
Whole wheat + 3.3 gm. wheat germ	0.30	1.49	2.22	40.0
Stabilized wheat germ	0.06	1.92	8.62	150–172
Country Home food				
10/ 2/35	0.68	1.49		277
10/24/35	0.82	1.49		229
11/18/35	1.01	1.49		183
12/19/35	0.99	1.49		183
1/ 9/36	0.96	1.49		205
2/ 7/36	1.68	1.49		108
4/13/36	2.13	1.49		98
4/17/36	1.71	1.49		128
				Ave. 176
Mooseheart food [1]				
3/3/37	1.90 gm.	1.92		63
3/4/37	0.78 gm.	1.92		224
3/5/37	0.79 gm.	1.92		112
3/6/37	0.87 gm.	1.92		138
3/7/37	0.95 gm.	1.92		112
3/8/37	1.13 gm.	1.92		101
3/9/37	0.84 gm.	1.92		153
				Ave. 129
Milk 3/4/37	5.9 cc.	1.92		84
Crystalline vitamin B	1.15 γ	1.92	452,500	200
Betaxin	1.9 γ	1.92	275,000	120
International standard absorbate	5.21 mg.	1.92	100	

[1] Total day's food, minus milk and bread, as weighed for sample servings.

are necessary before the level of vitamin B ingestion is able materially to affect appetite. During stabilized wheat germ and crystalline vitamin B periods, the vitamin B intake was increased about 50%. On the basis of the degree of stimulation obtained in this study, it would therefore seem that additional amounts of at least 150 units of vitamin B might be required to produce much effect upon appetite stimulation with young children.

A comparison of results on the basis of body surface area. Since the children in this study ranged in size from 15.7 to 45.8 kilograms, in order to compare results with individual children, the grams of food consumed per day have been calculated on a per kilogram basis. This treatment of the data, however, does not offer the best means of interpretation, since the amounts consumed per kilogram decrease with increases in weight. When the results are calculated on the basis of body surface area, a better comparison is possible between the younger and older children. The average grams of food per square meter of body surface for representative children of group I at the Country Home and group II at Mooseheart have been plotted in figure 1. From these charts it will be seen that the increases in vitamin B intake were able to produce increases in food consumption for nearly every child studied. There is more fluctuation in results between individual children, however, with the slightly older more normal girls observed at Mooseheart.

Relation of food consumption to weight gains. Since an increase in food consumption might be expected to produce gains in body weight, the changes in weight of the children have been analyzed. For this purpose the data for the Mooseheart girls have been presented (table 5) rather than that obtained at the Country Home, since it is believed that these girls were more capable of presenting normal responses. When results for the individual children were studied, however, it was observed that a direct relationship between grams of food consumed and gain in weight did not occur in all cases.

In order to explain this apparent lack of correlation between food consumption and weight gain, the caloric ingestion of group II is given in table 6, and certain data have been summarized for figure 2. It will be seen from these data that an increase in grams of food did not always mean an increase in calorie consumption. For example, the gram intake of the

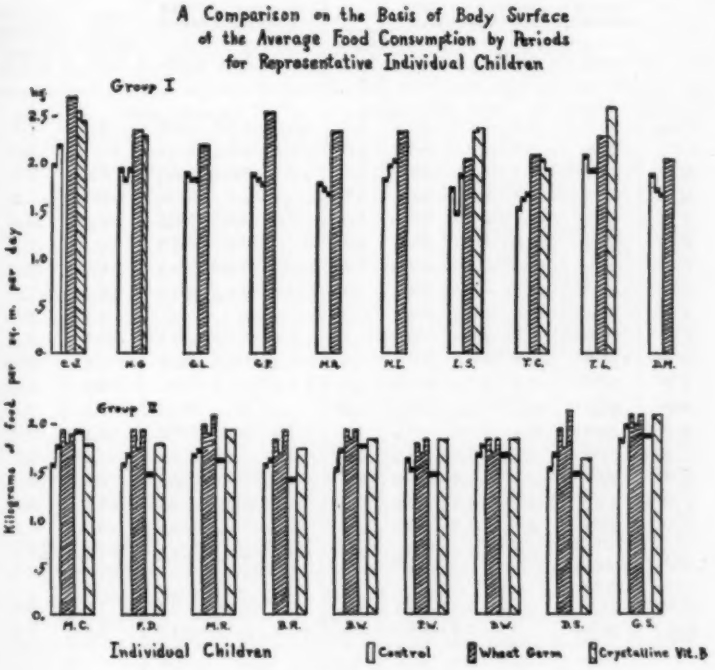
A Comparison on the Basis of Body Surface of the Average Food Consumption by Periods for Representative Individual Children
Group I
Group II
Kilograms of food per sq. m. per day
Individual Children
Control
Wheat Germ
Crystalline Vit. B

Figure 1

children during the January period was the highest of all periods, but the ratio of calories to grams in the food served was low for this period. Accordingly, the average calorie consumption was somewhat lower for this period, and weight gains were comparable to several other periods instead of being increased. The real effect of increased vitamin B intake

upon food consumption and weight is best seen in the November period. Here the grams served for first helpings and the caloric content of the food were nearly the same as the preceding period, but both the grams of food intake and the

TABLE 5

Weight changes of the girls in group II

	INITIAL WEIGHT	MONTHLY CHANGES CONTROL PERIOD			TOTAL GAIN FIRST 3 MO.	MONTHLY CHANGES WHEAT GERM PERIOD			TOTAL GAIN SECOND 3 MO.	GAIN FOR CRYSTALLINE VITAMIN B MONTH
	7/21	8/18[1]	9/15	10/13		11/10	12/8	1/5		
	kg.									
B.A.	30.5	−1.1	+1.1	+0.5	0.5	+0.7	+0.8	+0.9	2.4	1.5
C.C.	25.8	−0.7	+0.7	+1.0	1.0	+0.3	+0.4	....	..	1.0
M.C.	28.1	−1.1	+1.2	+0.9	1.0	+0.4	+0.3	−0.1	0.6	0.7
M.D.	32.8	−0.8	+2.2	+0.1	1.5	....	....	....	2.5	0.3
K.E.	28.8	−1.1	+1.7	+0.1	0.7	+0.2	−0.2	+0.5	0.5	0.6
F.D.	32.1	−0.6	+1.1	+0.3	0.8	+1.1	−0.3	+1.3	2.1	0.1
E.F.	24.0	−0.9	+0.8	+0.6	0.5	+0.7	−0.6	+0.1	0.2	0.6
E.G.	40.0	−0.7	+2.0	+0.5	1.8	+1.5	+1.4	−1.5[2]	1.4	1.7
A.G.	36.6	−0.2	+1.4	−0.3	0.9	+2.0	....	....	4.3	1.1
I.M.	32.9	−1.1	+1.3	−0.6	−0.4	+0.4	−1.0	+1.3	0.7	0.8
M.P.	29.8	−0.7	+1.6	+0.1	1.0	+1.2	−0.1	+0.5	1.6	0.6
A.P.	38.7	−1.1	+1.4	+1.0	1.3	+1.1	....	....	1.8	1.4
B.P.	25.6	−0.1	+1.3	+0.1	1.3	....	....	....	..	
M.R.	29.9	−0.4	+1.6	+0.4	1.6	+1.1	+0.1	+0.9	2.1	0.6
B.R.	29.6	−0.7	+1.3	−0.3	0.3	+1.3	−0.2	+0.8	1.9	0.7
B.W.	26.7	−0.9	+1.6	+0.2	0.9	+0.4	+0.6	−0.1	0.9	0.0
P.W.	33.1	−1.0	+1.1	+0.2	0.3	+1.1	+0.5	+0.3	1.9	0.5
D.W.	26.9	0	+1.3	+0.6	1.9	+0.7	−0.1	−0.8	−0.2	0.3
Ave.		−0.7	+1.4	+0.3	0.9	+0.9	+0.3	+0.3	+1.5	+0.8
	8/18									
G.S.	21.5	....	+0.8	+1.1	1.9	+0.5	+0.6	−0.1	1.0	0.6
D.S.	30.9	....	+1.9	+3.0	4.9	+1.4	+1.0	+1.3	3.7	1.4
	9/15									
J.B.	43.6	....	....	+1.3	...	−0.5	+0.4	+1.1	1.0	2.2
S.B.	39.1	....	....	−1.7	...	+1.4	−0.2	+1.0	+2.2	..

[1] Low weights because of strenuous exercise.

[2] Upper respiratory infection.

calorie intake per square meter of body surface were definitely increased. During this month the average weight gain for the group was the highest of any 4-week period being 0.9 kg. or 2 pounds.

Other factors, however, than the actual calorie consumption of the girls must be considered. When ages were examined it was noted that there was a slight correlation between

TABLE 6

Average calories of food consumed per day, per square meter, by each child, during each period

Group II

PERIOD DATE	1. 8/13–20	2. 9/9–15	3. 10/7–13	4. 11/4–11	5. 12/2–8	6. 12/30–1/5	7. 3/3–9	8. 3/31–4/6
DIET	No supplement			Stabilized wheat germ			Control	Crystalline vitamin B
B.A.	1572	1441	1837	2186	1850	....	....	1747
C.C.	1517	1842	2235	2253	2203	....	2208	2138
M.C.	1659	1699	2165	2077	2022	1653	2410	1930
M.D.	1596	1704	2044	....	1826	1536	2042	1937
F.D.	1735	1718	1783	2138	1806	1915	2054	1868
K.E.	1639	1526	1909	1910	1864	1774	....	2108
E.F.	1759	1516	2061	1961	1987	....	2151	2130
E.G.	1393	1404	1828	1956	2053	....	1968	1936
A.G.	1610	....	1821	....	1814	2060	2243	1964
I.M.	1515	1459	1854	1875	1728	1819	1934	1919
M.P.	1690	1675	1908	2163	1873	1531	2147	1800
A.P.	1377	1338	1474	1794	1685	1721	1769	2036
B.P.	1813	1686	2053	....	....	....	....	....
I.W.	....	....	....	1867	1857	1703	1927	2015
M.R.	1758	1596	2090	2099	2034	1946	2058	2115
B.R.	1703	1510	1840	1923	1765	1667	1981	1713
B.W.	1717	1576	2025	2170	2087	1725	2248	2077
P.W.	1742	1483	1784	1943	1904	1804	2016	1988
D.W.	1867	1601	2120	1984	1892	1685	2209	1959
D.S.	1590	1656	1802	2079	1858	1823	1935	1766
G.S.	1967	1817	2385	2188	2216	1991	2449	2074
J.B.	....	1369	1541	1553	1573	1596	1674	1635
S.B.	....	1232	1452	1694	1594	1529	1581	....
Ave.	1661	1564	1910	1991	1886	1749		
	1712			1875			1953	1945

greater weight gains occurring with the older girls. It is believed that a few of these older girls may have been approaching the age of puberty. The length of stay at Mooseheart also had an effect in that the girl presenting the greatest gain had just joined the group. It is thought that she might have

undergone a period less favorable for growth immediately before her entrance to Mooseheart. A possible explanation of the low gains obtained throughout the study might be the protein ingestion of the girls. During all periods the grams of protein intake ranged from only 2.13 to 2.51 gm. per kilogram of body weight, with an average of 2.27 gm. The 2.51 gm. were consumed during the November period of increased

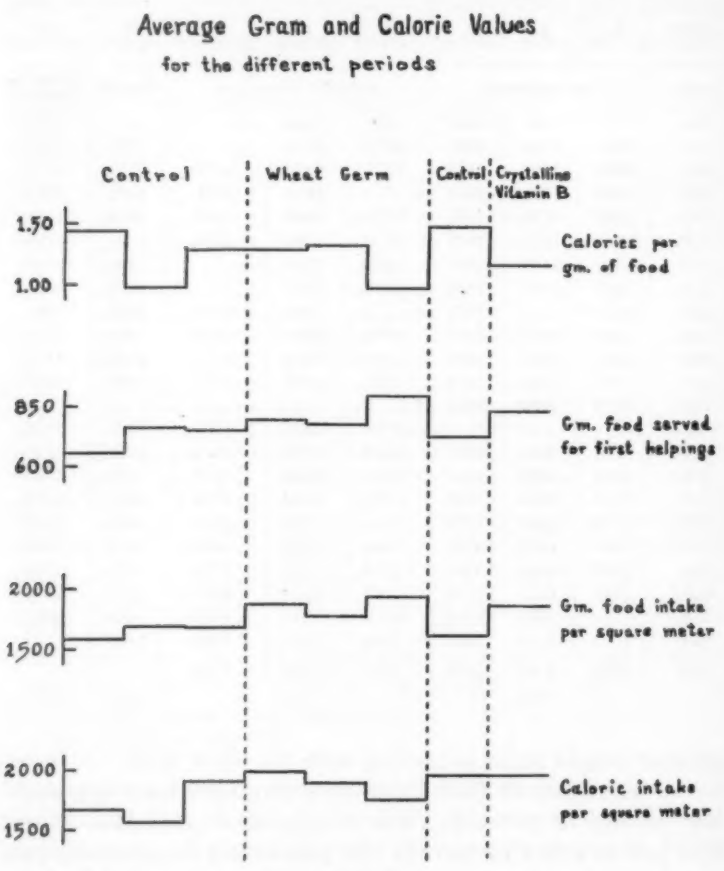

Figure 2

intake. Since Daniels et al. ('35) have presented data indicating that 3.2 gm. of protein per kilogram may be the optimum requirement, the low intake during the present study may be significant.

As a further check upon the results of the study, the complete market orders for group II for the first 6 months have been compared with market orders for a comparable group of girls for the preceding year. Examination of these data showed that the most significant difference between the two groups was the increased consumption of milk for group II during the 3 months they were receiving the stabilized wheat germ.

Other factors which may influence appetite. It is, of course, well known that appetite may be affected by many factors. To test some of these factors, during certain periods at the Country Home and at Mooseheart, records were kept of the primary choice of the children for cereals and of their desire for second servings.

In the primary selection of cereals, the girls at Mooseheart were allowed to vote on which of five cooked cereals they wished served. The results were as follows: malted wheat endosperm 110 votes, wheat endosperm 95, toasted whole wheat 74, oatmeal 47 and cracked wheat 45. It will be seen that in this primary selection of cooked cereals, the malted wheat cereal received 30% of the total votes.

When the results in regard to second servings were analyzed, it was observed that the least quantity of cereal was consumed during the period immediately after a time of cereal restriction. Thus, established habits would appear to be important in controlling appetite. The average results for the 4617 breakfasts which are included in the records indicated that in addition to habit such factors as flavor, consistency, and psychic appeal were important. A decided preference was expressed for the prepared cereals. Of the cooked cereals, those with more flavor or containing more of the whole grain were consumed with greater readiness. It is interesting to note that

of the refined cooked cereals, again the one with malted additions to the wheat endosperm was selected with most frequency.

SUMMARY

Factors affecting appetite have been studied through food consumption records which have been kept during 32 weeks for thirty-two children, age 4 to 10, at the Country Home for Convalescent Crippled Children, and during a similar period for twenty-two girls, age 9 to 11, at the Mooseheart Home for Child Training. Following control periods, the vitamin B intake of both groups was increased by means of: first, stabilized wheat germ, and second, crystalline vitamin B.

The vitamin B contents of the regular diets of the children averaged from 260 to 420 international standard units per day, while the supplements of wheat germ and crystalline vitamin B furnished from 120 to 200 additional units. A supplementary ingestion of approximately 150 units of vitamin B (representing an increase of about 50% in the daily vitamin B intake) produced increases of from 17 to 25% in the grams of food consumed per child per day.

The caloric ingestion during these periods of increased food consumption correlated to a slight degree with increased weight gains.

CONCLUSIONS

Since the higher levels of vitamin B administered during this investigation produced no apparent ill effects, did not force the growth, and did tend to stabilize the appetites of the children, it is concluded that the higher ingestions of vitamin B may be regarded as optimum.

LITERATURE CITED

DANIELS, A. L., M. K. HUTTON, E. M. KNOTT, O. E. WRIGHT, G. J. EVERSON AND F. SCOULAR 1935 A study of the protein needs of preschool children. J. Nutrition, vol. 9, p. 91.

EDITORIAL 1937 Utilization and retention of vitamin B_1 in children. J. Am. Med. Ass., vol. 109, p. 33.

GAYNOR, M. F., AND R. H. DENNETT 1934 Rice polish as a source of vitamin B complex. J. Pediat., vol. 4, p. 507.

KNOTT, ELIZABETH M. 1936 A quantitative study of the utilization and retention of vitamin B by young children. J. Nutrition, vol. 12, p. 597.

KNOTT, ELIZABETH M., AND FREDERIC W. SCHLUTZ 1937 Improvement and standardization of rat growth technic for short period assays of vitamin B. J. Biol. Chem., vol. 119, p. lviii.

MORGAN, A. F., AND M. M. BARRY 1930 Underweight children. Increased growth secured through the use of wheat germ. Am. J. Dis. Child., vol. 39, p. 935.

POOLE, M. W., B. M. HAMIL, T. B. COOLEY AND I. G. MACY 1937 Stabilizing effect of increased vitamin B_1 intake on growth and nutrition of infants: Basic study. Am. J. Dis. Child., vol. 54, p. 726.

ROSS, J. R., AND P. SUMMERFELDT 1935 Value of increased supply of vitamin B_1 and iron in the diet of children: Pater II. Am. J. Dis. Child., vol. 49, p. 1185.

SCHLUTZ, FREDERIC W., AND ELIZABETH M. KNOTT 1936 The use of a 10-day period for the assay of vitamin B by rat growth technic. J. Nutrition, vol. 12, p. 583.

SHERMAN, C., AND H. C. SHERMAN 1937 The vitamins. Am. Rev. Biochem., vol. 6, p. 335.

SUMMERFELDT, P. 1932 The value of an increased supply of vitamin B_1 and iron in the diet of children. Am. J. Dis. Child., vol. 43, p. 284.

STUDIES ON THE VITAMIN B_1 REQUIREMENTS OF GROWING RATS [1, 2]

AARON ARNOLD [3] AND C. A. ELVEHJEM

Department of Agricultural Chemistry, University of Wisconsin, Madison

TWO FIGURES

(Received for publication November 8, 1937)

The encouraging results we have obtained with chicks fed an autoclaved natural grain ration supplemented with graded levels of crystalline vitamin B_1 (Arnold and Elvehjem, '38) led us to try to adapt the ration for similar studies with rats. Several trials indicated that the autoclaved grain ration and the purified rat rations generally used were somewhat low in factor W (Elvehjem, Koehn and Oleson, '36; Frost and Elvehjem, '37). Since preparations containing this factor but deficient in vitamin B_1 were available at this laboratory, studies on a vitamin B_1 deficient purified rat ration were initiated. The results obtained indicate that it is possible to obtain normal rates of growth in rats when a suitably fortified vitamin B_1 deficient synthetic ration is supplemented with crystalline vitamin B_1 hydrochloride.

EXPERIMENTAL

Albino rats with an initial weight of 40 to 50 gm. were placed on experiment at about 25 days of age. The cages were equipped with raised wire screen bottoms (two meshes to the inch). Food and water were each supplied daily in glazed

[1] Published with the permission of the director of the Wisconsin Agricultural Experiment Station.

[2] Presented in part at the ninety-fourth meeting of the American Chemical Society at Rochester, September, 1937.

[3] Wilson and Company Fellow.

porcelain cups. Scattering of feed was minimized by the use of suitable glass covers on each cup. The covers contained openings of ½ inch diameter which permitted ready access to the ration. Food consumption was recorded daily. The animals were weighed weekly.

To determine whether the autoclaved natural grain ration which has proved to be successful in the chick assay procedure could be used with equal success in rat assays, the following studies were made.

Ration 240A developed by Kline, Keenan, Elvehjem and Hart ('32) served as the basal ration for the rats. It has the following composition:

Autoclaved portion:	
Ground yellow corn	59
Pure flour middlings	25
Crude domestic acid precipitated casein	12
Untreated portion:	
$CaCO_3$ (precipitated)	1
$Ca_3(PO_4)_2$ (precipitated)	1
Iodized salt (0.02% potassium iodide)	1
Cod liver oil	1

The rats fed the unsupplemented basal ration (fig. 1, group 47) gained in weight for 2 or 3 weeks and then remained practically constant in weight for a number of weeks. The food intake of the animals was low and after 4 to 6 weeks on the ration the animals exhibited a vitamin B_4 deficiency. The backs of the animals were hunched, the jowls protruded and the animals walked high on their rear legs with ataxia. The symptoms of polyneuritis appeared 2 to 4 weeks later. These results have been discussed elsewhere (Elvehjem and Arnold, '36). Control groups which received supplements of 2.0 (group 48), 2.5 (group 49), or 3.0 (group 50) % of standard yeast 3 (10 I.U. vitamin B_1 per gram) grew moderately well during the 6-week experimental period.

To induce a greater food intake by the animals in the early stages of the experiment, the ration was modified to contain greater amounts of other nutritive factors. Four per cent

autoclaved yeast[4] was added as a source of the heat stable vitamin B complex. Ten per cent hulled peanuts was added since this material supplies considerable vitamin B_4 (Kline, Bird, Elvehjem and Hart, '36). Additional amounts of unsaturated fatty acids were also supplied by this product. In

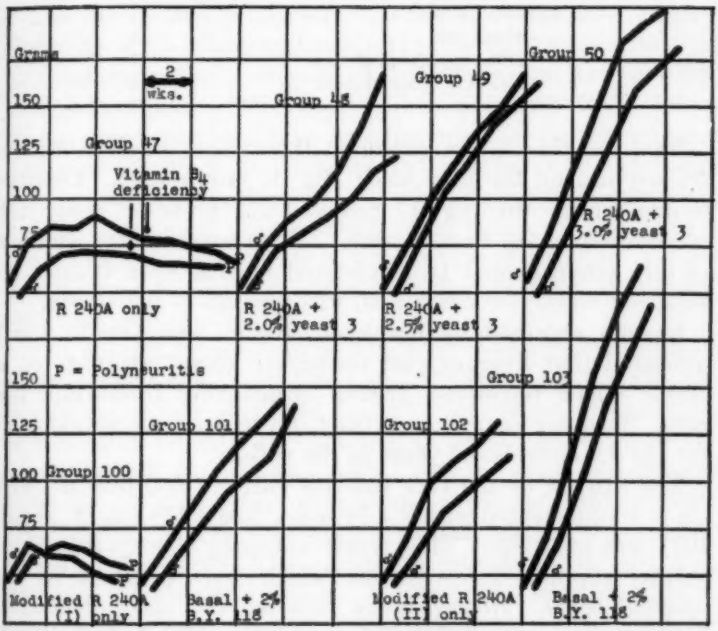

Fig. 1 Individual growth records of rats fed ration 240A and modifications of the autoclaved grain ration.

addition to the above supplements the effect of the addition of certain animal tissues was also tested. In one case autoclaved lung was added and the flour middlings were omitted in order to keep the protein level of the ration constant. The modified ration had the following composition.

[4] Strain C, Anheuser-Busch, St. Louis.

Modified ration 240A (I)

Autoclaved portion:	
Ground yellow corn	70
Crude domestic acid precipitated casein	12
Hulled peanuts	5
Yeast[6]	4
Vacuum desiccated lung[6]	5
Untreated portion:	
Iodized salt	1
$CaCO_3$ (precipitated)	1
$Ca_3(PO_4)_2$ (precipitated)	1
Cod liver oil	1

Rats fed this ration exhibited the typical vitamin B_1 deficiency during the fifth week (fig. 1, group 100). Positive control group 101 (fig. 1) received 2% brewers' yeast 118 in addition to the basal ration. The growth of the animals in this group cannot be considered normal even though an adequate supply of vitamin B_1 was present in the ration.

Results obtained by Elvehjem, Koehn and Oleson ('36) indicated that liver extract contained liberal amounts of a factor which possessed growth stimulating properties for rats. Two per cent liver extract, therefore, was substituted for the 4% autoclaved yeast in the ration.

The growth of the rats fed this ration (modified R 240A (II)) supplemented with 2% brewers' yeast 118 (fig. 1, group 103) was excellent. This result indicates clearly that liver extract supplied those factors which were deficient in the original basal. However, the rats fed the unsupplemented basal ration continued to grow slowly. The liver extract, therefore, added sufficient vitamin B_1 to protect the rats from polyneuritis and even permitted some growth to occur.

Since the autoclaved grain ration did not appear to be suitable for vitamin B_1 studies with rats unless considerably modified, a new ration was developed. This ration (ration 112) has the following composition:

[6] See footnote 4, page 431.

[6] Wilson Laboratories, Chicago.

Sucrose	62
Purified casein	18
Autoclaved peanuts	10
Autoclaved yeast [7]	10
Salts I	4
Factor W	≅ 2 liver extract
Halibut liver oil for vitamins A and D	2 drops twice weekly

The casein was prepared from diluted skim milk by precipitation with dilute hydrochloric acid, and further purified by solution in dilute ammonium hydroxide, and precipitation with dilute acid. This process was repeated three times. Hulled fresh peanuts have been included in the ration as a source of vitamin B_4 and unsaturated fatty acids. The peanuts were ground and autoclaved for 5 hours at 15 pounds pressure before being used. Autoclaved yeast [7] was used to supply the heat stable components of the vitamin B complex. Salts I (Phillips and Hart, '35; Kline, Bird, Elvehjem and Hart, '36) was used throughout.

The growth stimulating principle of liver extract has been designated factor W by Frost and Elvehjem ('37). The following procedure was used for the preparation of this fraction free of vitamin B_1: 100 gm. of liver extract were dissolved in 200 cc. of water and 1 liter of ethyl alcohol and 1200 cc. of ethyl ether were added with constant stirring. The precipitate was allowed to settle out and the supernatant liquid siphoned off. The precipitate was dissolved in 100 cc. of water and reprecipitated. After being washed with an ether-alcohol-water mixture, the precipitate was dissolved in 400 cc. of water and allowed to stand for 24 hours, whereupon a light colored precipitate settled out, which was centrifuged off and washed with 25 cc. of water. The combined filtrate and washings were concentrated in vacuo at 50°C. to 80 cc. Upon the addition of 10 volumes of acetone, a dark gummy precipitate formed. The precipitate was dissolved in 50 cc. of water and reprecipitated with 10 volumes of acetone. After being washed with acetone, the precipitate

[7] See footnote 4, page 431.

was dissolved in 250 cc. of water and allowed to stand, whereupon more insoluble material settled out, which was centrifuged off and washed with 25 cc. of water. The combined filtrate and washings were diluted to 300 cc. with alcohol and stored at low temperature for use.

The procedure used in making up the rations was to dry the water solution of factor W on the casein in the drying room (50°C.) before a fan. Supplements to be added to the ration which were also contained in solution, such as vitamin concentrates, were then dried in vacuo over calcium chloride on weighed portions of the dry purified casein-factor W component of the diet. The sucrose, autoclaved yeast and autoclaved peanuts were combined in the proper proportions and ground in a Burr mill before use.

The results obtained when the rats were placed on the basal alone or on the basal plus vitamin B_1 supplements at weaning are given in table 1. Rats fed the unsupplemented basal ration showed the polyneuritic syndrome in about 5 to 6 weeks. Supplements of 0.3% brewers' yeast 131,[8] 0.2% international standard or 60 micrograms of crystalline vitamin B_1 hydrochloride [9] (natural source) per 100 gm. of ration 112 resulted in average growth rates of 3.0 to 3.2 gm. per day for the 6-week experimental period. A lower growth rate resulted when rats were fed 50 micrograms of vitamin B_1, obtained as a concentrate from natural sources, per 100 gm. of ration 112. The average daily gain of these rats was 2.8 gm. for the 6-week period. An increase of the same vitamin B_1 supplement to 100 micrograms per 100 gm. of ration 112 resulted in a much improved rate of gain amounting to approximately 3.8 gm. daily. The responses in growth of the rats when they were fed ration 112 supplemented with sources of vitamin B_1 such as brewers' yeast 131, the international standard or as the vitamin itself show that ration

[8] We are indebted to Dr. Harold Levine, of the Premier-Pabst Corporation, Milwaukee, for several generous samples of yeast.

[9] The crystalline vitamin B_1 hydrochloride samples were kindly weighed out by Mr. H. A. Campbell on a micro-analytical balance.

TABLE 1

Growth of rats fed ration 112 alone or ration 112 supplemented with vitamin B_1 from brewers' yeast 131, the international standard adsorbate or vitamin B_1 preparations for a 6-week experimental period

SUPPLEMENT TO RATION 112	RAT NUMBER	INITIAL WEIGHT	WEIGHT 6 WEEKS	DAILY GAIN
		gm.		
None			Polyneuritis	
	102–21	37	20 days	
	102–22	58	28	
	102–23	56	24	
	104–71	50	46	
	104–72	50	46	
	108–61	52	46	
	108–62	49	55	
	118–90	39	41	
	118–00	43	47	
Average	9 ♂	48	39	
			gm.	gm.
0.3% brewers' yeast 131	108–41	49	202	
	108–42	54	174	
	109–21	50	191	
	109–22	49	155	
	118–85	41	185	
Average	5 ♂	49	181	3.2
0.2% international standard	109–01	48	165	
	109–02	48	173	
	118–73	37	170	
Average	3 ♂	44	169	3.0
0.00006% crystalline vitamin B_1 (natural source)	118–05	38	160	
	118–95	40	178	
Average	2 ♂	39	169	3.1
0.00005% vitamin B_1 (natural source)	106–61	42	168	
	106–62	42	168	
	108–71	52	163	
	108–72	47	166	
	109–81	49	136	
	109–82	49	170	
Average	6 ♂	47	165	2.8
0.00010% vitamin B_1 (natural source)	108–81	50	192	
	108–82	44	215	
	108–83	44	210	
Average	3 ♂	46	206	3.8

112 is practically complete in all nutrient essentials for rats aside from vitamin B_1.

To obtain greater differences between the groups receiving graded levels of vitamin B_1, the animals were depleted of the major part of their vitamin B_1 reserves before they were given their supplements. The results given in table 2 indicate that rats restricted to the basal ration for a 10- to 14-day depletion period required a higher level of vitamin B_1 in the ration to make growth gains comparable to those reported in table 1. Extensive comparisons are not possible since only the international standard is common to both tabulations. It is interesting to note, however, that the ratio of the international standard to vitamin B_1 is approximately the same in both tables. Comparable growth gains resulted when the rats were fed ration 112 supplemented with 0.2% international standard or 60 micrograms of crystalline vitamin B_1 (natural source) per 100 gm. of ration (table 1). Approximately equal growth responses resulted when the rats were fed ration 112 supplemented with 0.2% international standard or 60 micrograms of synthetic crystalline vitamin B_1 hydrochloride (sample 2) per 100 gm. of ration (table 2). Another comparison may be made. The growth responses which resulted when the rats were fed ration 112 supplemented with 0.25% brewers' yeast 132 or 60 micrograms of vitamin B_1 hydrochloride per 100 gm. of ration (table 2) are approximately the same. An increase of one-third in each of these vitamin B_1 sources resulted in equal growth responses. The average daily gains which resulted when the rats were fed ration 112 supplemented with 0.33% brewers' yeast 132 or 80 micrograms of vitamin B_1 hydrochloride per 100 gm. of ration are also comparable. When the rats were fed 100 micrograms of vitamin B_1 (tables 1 and 2) comparable growth gains resulted which indicated that this amount of vitamin B_1 in the ration was close to the optimum value.

In view of the good rates of growth reported here it is interesting to examine the vitamin B_1 intake of the animals that were fed ration 112 supplemented with crystalline vitamin B_1 hydrochloride. The values were derived from weekly

TABLE 2

Growth of rats fed the basal ration for a 10- to 14-day depletion period, then fed ration 112 alone or ration 112 supplemented with vitamin B_1 from brewers' yeast 132, the international standard adsorbate or vitamin B_1 hydrochloride (sample 2) for a 5-week experimental period

SUPPLEMENT TO RATION 112	RAT NUMBER	INITIAL WEIGHT	WEIGHT 5 WEEKS	DAILY GAIN
		gm.		
None			Polyneuritis	
	120–11	33	34 days	
	120–21	61	51	
	123–72	51	35	
	123–81	52	35	
	126–03	62	56	
	126–06	61	47	
	126–12	66	56	
	127–62	74	34	
	127–71	71	28	
Average	9 ♂	59	42	
			gm.	*gm.*
0.25% brewers' yeast 132	120–33	68	106	
	126–05	64	150	
	126–25	66	172	
	126–33	58	158	
	127–86	56	144	
Average	5 ♂	62	146	2.4
0.33% brewers' yeast 132	126–14	62	160	
	126–26	64	152	
	126–32	58	206	
Average	3 ♂	61	173	3.2
0.2% international standard	123–65	64	125	
	123–94	66	165	
	126–01	66	200	
	126–15	65	125	
	126–16	74	215	
	127–61	84	170	
	127–74	81	182	
	127–87	62	138	
Average	8 ♂	70	165	2.7
0.00006% vitamin B_1 (sample 2)	120–12	55	129	
	122–54	56	104	
	126–11	70	186	
	126–31	65	190	
	127–73	82	168	
Average	5 ♂	66	155	2.5
0.00008% vitamin B_1 (sample 2)	123–91	61	166	
	127–63	94	205	
	127–75	88	204	
Average	3 ♂	81	192	3.2
0.00010% vitamin B_1 (sample 2)	126–21	69	186	
	126–23	62	176	
	126–24	66	215	
Average	3 ♂	66	192	3.6

food consumption records. The data charted in figure 2 give a comparison of the average daily intakes of vitamin B_1 hydrochloride of the animals that received 60, 80 or 100 micrograms of crystalline vitamin B_1 hydrochloride (sample 2) per 100 gm. of ration 112. The average daily consumption progressively increased from 3.8 to 5.7 micrograms of vitamin B_1 hydrochloride when the ration contained the lowest level

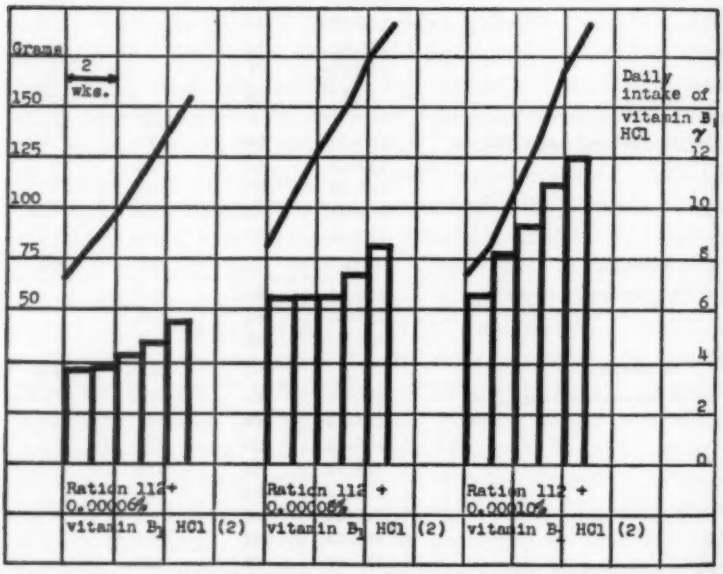

Fig. 2 Average daily intake of the vitamin B_1 hydrochloride of rats fed ration 112 supplemented with 60, 80 or 100 micrograms of vitamin B_1 hydrochloride (sample 2) per 100 gm. of ration.

of vitamin B_1, from 6.6 to 8.6 micrograms of vitamin B_1 hydrochloride when it contained the intermediate level and from 6.7 to 12.7 micrograms of vitamin B_1 hydrochloride when it carried the highest level of vitamin B_1.

The figures obtained from food consumption records may also be used to determine the vitamin B_1 ingested per kilogram body weight per day. The values were calculated from

the records of the rats which received 100 micrograms of vitamin B_1 hydrochloride per 100 gm. of ration 112 and are calculated on the basis of the average weights of the animals each week as determined by the weights at the beginning and end of the 7-day period. The results given in table 3 are expressed in terms of the crystalline vitamin B_1 hydrochloride and its international unit equivalency (3 micrograms of vitamin B_1 hydrochloride (sample 2) $\cong$ 1 international unit) on the basis of previous data (Arnold and Elvehjem, '38).[10]

TABLE 3

Daily intake of vitamin B_1 hydrochloride (sample 2) expressed in terms of body weight. Record obtained from rats that received 100 micrograms of vitamin B_1 hydrochloride per 100 gm. of ration 112

WEEK	MEAN WEIGHT	FOOD CONSUMPTION TOTAL FOR WEEK	VITAMIN B_1 · HCl INGESTED PER DAY	VITAMIN B_1 · HCl INGESTED PER KILOGRAM BODY WEIGHT PER DAY	
	gm.	*gm.*	*micrograms*	*micrograms*	*international units*
1	74	47	6.7	91	30
2	94	58	8.3	88	29
3	122	66	9.4	77	26
4	152	76	11.0	72	24
5	180	85	12.1	68	23

DISCUSSION

The results submitted here demonstrate quite clearly that while symptoms of vitamin B_1 deficiency can be produced in rats on a variety of vitamin B_1 low rations, normal growth can be obtained through the addition of pure preparations of the vitamin only when the basal ration contains an adequate supply of the other essential factors. Certain difficulties may be encountered even in the production of uncomplicated polyneuritis if the basal ration is too low in certain essential

[10] Several different samples of the crystalline synthetic vitamin have been used and some variation in potency has been observed. However, in our preliminary runs no attempt was made to check the moisture content of the crystals before use. Our purpose was to study the effect of the addition of the crystalline material to the basal ration rather than the variation in potency of individual samples. Studies on the comparison of the potency of different samples are now in progress.

factors and contains appreciable amounts of vitamin B_1. In this case a prolonged feeding period is required and other deficiencies develop before the animal is completely depleted of the anti-neuritic factor.

Although it is difficult to set a definite figure for the normal growth rate of rats, a growth approaching 4.0 gm. per day must be considered very good. Very recently Edgar and Macrae ('37) obtained a daily growth of 4.3 to 4.6 gm. over a 4- to 6-week period for male rats on a purified ration supplemented by the daily administration of 50 micrograms of riboflavin, 10 to 20 micrograms of vitamin B_1 hydrochloride, fullers' earth filtrate $\cong 1$ gm. of yeast and fullers' earth eluate $\cong 2$ gm. yeast. The properties of the fullers' earth eluate factor agree in many points with those for vitamin B_6 (György, '35 a, b; Harris, '35; Birch and György, '36; Lepkovsky, Jukes and Krause, '36) and the fullers' earth filtrate factor with those for factor W (Elvehjem, Koehn and Oleson, '36, and Frost and Elvehjem, '37) and the filtrate factor of Lepkovsky and Jukes ('36). The high levels used by Edgar and Macrae probably compensated for any destruction of the above factors which may have taken place during the autoclaving of the yeast extract which they used for the preparation of their concentrates.

The lack of a good source of factor W was probably the reason for the reduced growth rates observed by Bender and Supplee ('37). These investigators supplied 10 micrograms of riboflavin, 100 mg. of autoclaved rice polishings concentrate and 12.5 micrograms vitamin B_1 hydrochloride daily to rats on a purified diet. The average daily growth rate was approximately 1.25 gm. over a 42-day period. When the riboflavin supplement was increased to 20 micrograms daily, the average growth of the rats was approximately 1.7 gm. per day during a 42-day period. These investigators suggested that their basal ration might not supply sufficient amounts of other factors not well differentiated at that time. Waterman and Ammerman ('35) observed an average daily growth rate (males) of approximately 2.2 gm. when fed the basal ration

supplemented with 10 micrograms of vitamin B_1 hydrochloride daily. When the vitamin B_1 hydrochloride supplement was increased to 20 micrograms daily the average daily growth (males) was approximately 2.9 gm. over a 45-day period. Since the latter investigators used 15 parts of autoclaved yeast in the diet, it appears that this source cannot always be depended upon to supply adequate amounts of factor W.

The results submitted here indicate that the addition of an adequate amount of factor W to a vitamin B_1 deficient basal ration reduced the vitamin B_1 requirements of the rats. The animals fed 60 micrograms of vitamin B_1 hydrochloride per 100 gm. of ration 112 obtained an average of 3.8 micrograms of vitamin B_1 hydrochloride daily during the first week of the experiment. The daily food consumption increased during the course of the experimental period so that during the last week on experiment the rats ingested an average of 5.7 micrograms of vitamin B_1 hydrochloride daily. The average growth for this group was 2.5 gm. per day. Similarly the vitamin B_1 intake of the rats fed 80 micrograms of vitamin B_1 hydrochloride per 100 gm. of ration 112 rose from an average of 6.6 to 8.6 micrograms of vitamin B_1 hydrochloride daily during the course of the 5-week experimental period which resulted in an average growth rate of 3.2 gm. per day for the period. The vitamin B_1 ingested by the rats fed 100 micrograms of vitamin B_1 hydrochloride per 100 gm. of ration 112 increased from an average of 6.7 micrograms of vitamin B_1 hydrochloride daily to 12.1 micrograms daily during the experimental period. The average daily growth for this group was 3.6 gm. per day. Calculations based on food consumption records show that the vitamin B_1 hydrochloride ingested by the rats in the last group decreased from 30 international units of 23 international units per kilogram of body weight per day during the course of the experimental period. It is impossible to establish the exact optimum vitamin B_1 intake per kilogram of body weight for rats since the experiments have not been continued for extended periods of time.

Although the results submitted here are encouraging we do not propose that the rat growth method is sufficiently developed for assay purposes. The data yield definite information regarding the vitamin B_1 requirements for normal growth. Ration 112 appears to contain ample amounts of essential nutritive factors other than vitamin B_1. The procedure to be used for assay purposes, however, must depend on more extensive collaborative observations.

SUMMARY

1. The autoclaved grain ration (ration 240A) cannot be used for studies on vitamin B_1 with rats unless it is considerably modified.

2. A synthetic vitamin B_1 low ration (ration 112) has been developed which includes several recently disclosed factors of the vitamin B complex.

3. Rats restricted to ration 112 supplemented with 80 to 100 micrograms of vitamin B_1 hydrochloride per 100 gm. of ration grow at a normal rate.

LITERATURE CITED

ARNOLD, A., AND C. A. ELVEHJEM 1938 Studies on the vitamin B_1 requirements of growing chicks. J. Nutrition, vol. 15, p. 403.

BENDER, R. C., AND G. C. SUPPLEE 1937 Significance of quantitative relationships on vitamin B complex studies. J. Am. Chem. Soc., vol. 59, p. 1178.

BIRCH, T. W., AND P. GYÖRGY 1936 A study of the chemical nature of vitamin B_2 and methods for its preparation in a concentrated state. Biochem. J., vol. 30, p. 304.

EDGAR, C. E., AND T. F. MACRAE 1937 Water soluble vitamins. VIII. Essential dietary factors for the rat present in autoclaved yeast extracts in addition to lactoflavin. Biochem. J., vol. 31, p. 886.

ELVEHJEM, C. A., AND A. ARNOLD 1936 Interrelationships of vitamins. Nature, vol. 137, p. 109.

ELVEHJEM, C. A., C. J. KOEHN, JR., AND J. J. OLESON 1936 A new essential dietary factor. J. Biol. Chem., vol. 115, p. 707.

FROST, D. V., AND C. A. ELVEHJEM 1937 Further studies on factor W. J. Biol. Chem., vol. 121, p. 255.

GYÖRGY, P. 1935 a Investigations on the vitamin B_2 complex. I. The differentiation of lactoflavin and the 'rat anti-pellagra' factor. Biochem. J., vol. 29, p. 741.

———— 1935 b Investigations on the vitamin B_2 complex. II. The distribution of lactoflavin and of the 'pellagra preventing' factor (vitamin B_6) in natural products of animal origin. Biochem. J., vol. 29, p. 767.

HARRIS, L. J. 1935 Flavin and the pellagra-preventing factor as separate constituents of a complex vitamin B_2. Biochem. J., vol. 29, p. 776.

KLINE, O. L., H. R. BIRD, C. A. ELVEHJEM AND E. B. HART 1936 An improved synthetic ration for vitamin B_6 studies. J. Nutrition, vol. 11, p. 515.

KLINE, O. L., J. A. KEENAN, C. A. ELVEHJEM AND E. B. HART 1932 The use of the chick in vitamin B_1 and B_2 studies. J. Biol. Chem., vol. 99, p. 295.

LEPKOVSKY, S., AND T. H. JUKES 1936 The effect of some reagents on the 'filtrate factor' (a water-soluble vitamin belonging to the vitamin B complex and preventing a dietary dermatitis in chicks). J. Biol. Chem., vol 114, p. 109.

LEPKOVSKY, S., T. H. JUKES AND M. E. KRAUSE 1936 The multiple nature of the third factor of the vitamin B complex. J. Biol. Chem., vol. 115, p. 557.

PHILLIPS, P. H., AND E. B. HART 1935 The effect of organic dietary constituents upon chronic fluorine toxicosis in the rat. J. Biol. Chem., vol. 109, p. 657.

WATERMAN, R. E., AND M. AMMERMAN 1935 Studies of crystalline vitamin B. V. The effect of graduated doses on growing rats. J. Nutrition, vol. 10, p. 35.

THE RELATION OF THE 'GRASS JUICE FACTOR' TO GUINEA PIG NUTRITION[1]

G. O. KOHLER, C. A. ELVEHJEM AND E. B. HART
Department of Agricultural Chemistry, University of Wisconsin, Madison

FIVE FIGURES

(Received for publication November 24, 1937)

Earlier work in this laboratory (Elvehjem, Hart, Jackson and Weckel, '34) demonstrated a seasonal variation in the nutritive value of mineralized milks. Rats fed a mineralized milk, produced by cows on good pasture, grew at a rate of 4 to 4.5 gm. per day during a 6-week experimental period. When milk, produced by cows receiving a winter barn ration, was used as the basal diet, growth of only 2 to 2.5 gm. per day was obtained. Later it was demonstrated (Kohler, Elvehjem and Hart, '36) that the addition of fresh lawn clippings or the pressed juice from grass to the mineralized winter milk produced a rate of growth comparable to that obtained on summer milk.

In a more recent publication (Kohler, Elvehjem and Hart, '37) results were presented to show that various supplements rich in the known vitamins produced little or no growth response in rats on a basal winter milk diet. This led to the conclusion that the growth stimulating factor of grass was distinct from all the known vitamins. At this time it was pointed out that considerable variation was encountered in different groups of rats when placed on the basal ration, presumably due to differences in the storage of certain factors in the young rats when started on the experiment. Since

[1] Published with the permission of the director of the Wisconsin Agricultural Experiment Station.

guinea pigs are herbivorous, we felt that this species might be a more suitable animal for the assay of a factor which is found most abundantly in fresh green plant tissue.

The present paper deals with the growth of guinea pigs on mineralized winter milk and the growth-promoting action of supplements similar to those used in our earlier work with rats.

EXPERIMENTAL

Two preliminary experiments were made in order to study the reaction of guinea pigs to diets made up largely of whole milk. The milk used was obtained fresh each morning from the same cow (Holstein) at the dairy barn. The whole milk was fed twice daily in sufficient quantities to allow ad libitum consumption. In addition to the milk each guinea pig received a daily mineral supplement consisting of 1 mg. of Fe as ferric pyrophosphate, 0.1 mg. of Cu as copper sulfate, and 0.1 mg. Mn as manganese sulfate. The salts were incorporated into a small amount of dextrin and fed in a small gelatin capsule. The animals were weighed daily.

In the first trial four guinea pigs weighing about 300 gm. each were used. Two pigs were placed on the basal mineralized milk alone, one on the basal plus 2 cc. of orange juice daily and one on the basal plus 5 gm. of fresh oat grass which had been grown in the greenhouse.

For the second experiment six guinea pigs were divided into three groups of two pigs each. Two pigs received the basal mineralized milk diet and the four remaining pigs were given the same diet except that the milk was aerated for 1 hour at 60 to 65°C. Two of the pigs receiving the aerated milk were given 1 mg. of ascorbic acid per animal daily. The aerated milk was used to determine if a variation in the vitamin C intake would alter the results. Growth curves for the ten pigs are given in figure 1.

The sequence of events observed when the pigs were placed on the milk diets was very similar in each animal. During the first week or two the animals lost considerable weight. After

this preliminary period, they apparently became accustomed to the liquid diet and consumed fairly large amounts of milk. For the next few weeks they remained at constant weight or in some cases gained slightly until their original losses were regained. Between the fourth and seventh weeks the animals began to lose weight rapidly, some losing as much as 70 to 80 gm. in 1 week. At this stage respiratory trouble was noted in most cases. Immediately preceding death, clonic contractions of the legs were observed.

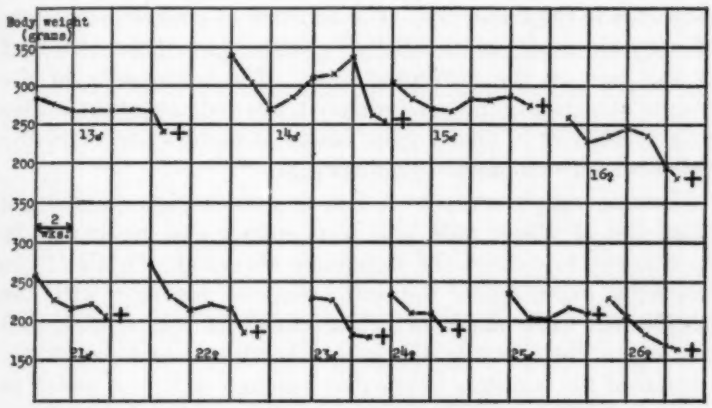

Fig. 1 Weight records of guinea pigs receiving mineralized winter milk (ad lib.). Nos. 13, 14, 25 and 26 received whole raw milk. No. 15 received raw milk + 5 gm. greenhouse oat grass daily. No. 16 received raw milk + 2 cc. orange juice daily. Nos. 21 and 22 received aerated milk. Nos. 23 and 24 received aerated milk + 1 mg. pure vitamin C daily.

Autopsies were made on all the animals and no signs of scurvy or rickets could be detected. In many cases the lungs showed advanced states of inflammation and congestion and, in some animals, necrotic areas in the lungs were evident. Since all animals showed a temporary favorable response on the milk after the first week or two, it appears that the animals actually suffered from a dietary deficiency which brought on the extreme debility and susceptibility to infection.

Since the addition of orange juice or oat grass did not alter the changes observed, it is obvious that neither vitamin C nor vitamin P (Armentano et al. '36) were limiting factors. The oat grass was tested on rats simultaneously and found to be inactive as a source of the 'grass juice factor.' Thus the failure of this oat grass to stimulate growth in guinea pigs did not eliminate the importance of this factor in the nutrition of guinea pigs.

The animals receiving the aerated milk died a little sooner than the other pigs. However, no signs of scurvy were evident even in these animals. The addition of generous amounts of ascorbic acid gave no better results than those observed in the pigs on the untreated milk. The uniformity of the results obtained in the preliminary trials indicates that winter milk is deficient in one or more essential factors necessary for the normal development of guinea pigs.

Various supplements were fed to guinea pigs receiving a basal diet of winter milk plus iron, copper and manganese in an attempt to correct the deficiency observed. In all of the following experiments the salt solutions together with the supplement were placed in a clean dish with a little milk each morning. Later in the day, when the animal had completely consumed the contents of the dish, enough milk was added to insure ad libitum feeding. Since milk contains 2 to 2.5 mg. of vitamin C per 100 cc., and the guinea pigs consumed 80 to 200 cc. of milk per day, there should be no danger of an inadequate supply of this vitamin. Unpublished work in our laboratory has shown that 0.3 mg. of pure ascorbic acid per day is sufficient to prevent the onset of scurvy and to support a good rate of growth. However, as a precautionary measure all animals were given ½ cc. of orange juice daily by pipette.

In figure 2 are presented growth curves of guinea pigs which received a series of supplements chosen to supply ample quantities of various known vitamins. The supplements were as follows: 5 gm. whole wheat meal, 5 gm. white cornmeal, 2 gm. brewers' yeast, 1 gm. vacuum dried whole

liver (pork),[2] 0.5 gm. liver extract powder,[2] and 0.5 gm. 92% alcohol soluble liver extract.[2] The wheat and cornmeal were found to be ineffective at the original levels, but when 7.5 gm. per day were fed, fair rates of growth were obtained. The vacuum dried whole liver gave some growth when the level was increased to 1.5 gm. per day, but neither of the liver extracts was effective. Apparently, the active principle of the liver is not extracted, or is destroyed by the commercial

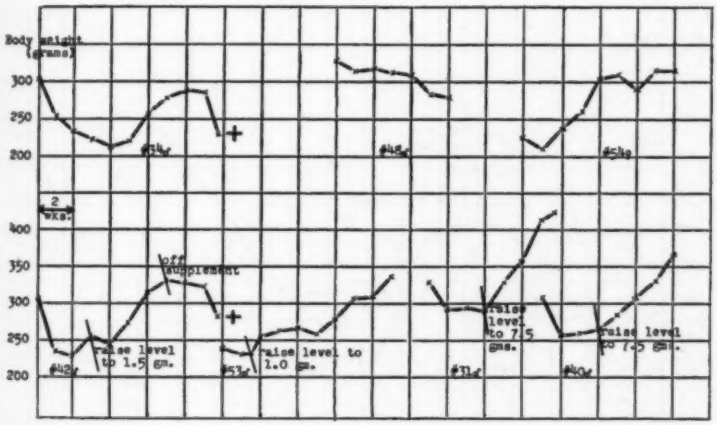

Fig. 2 Weight records of guinea pigs receiving mineralized winter milk (ad lib.) plus supplements containing liberal quantites of known vitamins. The daily supplements were as follows: No. 34, control; no. 48, 0.5 gm. 92% alcohol-soluble liver extract; no. 54, 2.0 gm. brewers' yeast; no. 42, 1.0 gm. vacuum dried whole liver; no. 53, 0.5 gm. liver extract powder; no. 31, 5.0 gm. whole wheat meal; no. 40, 5.0 gm. white cornmeal. All received 0.5 cc. orange juice daily.

processes used in the preparation of liver extracts. The inferior response given by the yeast together with the remarkable growth produced by grasses, shown in figure 3, adds credence to the hypothesis that the 'grass juice factor' described for rats is the same as the limiting factor concerned here.

[2] We are indebted to Dr. David Klein, Wilson Laboratories, Chicago, for the samples of liver and liver extract used.

The effects of feeding dried grasses as supplements to guinea pigs on the mineralized winter milk are shown in figure 3. The three cereal grasses (nos. 462, 458, 455)[3] were produced under comparable conditions. They were grown at the same time on plots of the same field so that they were exposed to identical weather conditions. They were cut at the same stage of growth, 1 month after emergence, and were dried at 82°C. for 4 hours.

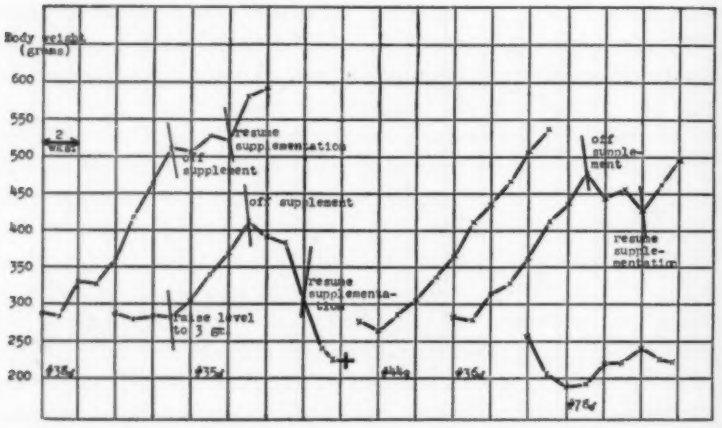

Fig. 3 Weight records of guinea pigs receiving mineralized winter milk (ad lib.) plus dehydrated grasses. The daily supplements were as follows: no. 38, 2.0 gm. barley grass no. 462; no. 35, 2.0 gm. oat grass no. 455; no. 44, 2.0 gm. barley grass no. 462; no. 36, 2.0 gm. wheat grass no. 458; no. 78, control. All received 0.5 cc. orange juice daily.

The barley grass, which was the most effective, produced a growth rate of 5.3 gm. per day from the second to the seventh week of the experiment. The wheat grass was only slightly less potent than the barley grass. However, in the case of the oat grass, it was necessary to raise the level to 3 gm. per day to produce good growth.

[3] These grass samples were generously supplied by Mr. C. F. Schnabel of the American Butter Company.

After 7 weeks on experiment, pigs nos. 35, 36 and 38 were taken off the supplement and fed the mineralized milk alone. Growth stopped almost immediately. After 3 weeks on the basal ration alone, during which period these animals actually lost weight, supplementation was resumed. Once again remarkable growth resulted in the animals receiving the barley and wheat grasses (pigs nos. 36 and 38). The animal receiving the oat grass (pig no. 35) was in such poor condition

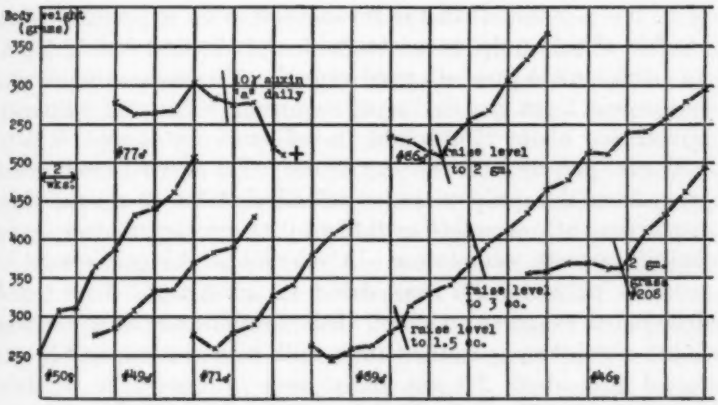

Fig. 4 Weight records of guinea pigs receiving mineralized winter milk (ad lib.) plus various preparations of grass or auxin. The daily supplements were as follows: no. 50, 20 gm. fresh lawn clippings; no. 49, 20 cc. juice pressed from lawn clippings; no. 71, 1.0 gm. frozen oat grass pulp no. 207 (batch 1); no. 89, 0.75 cc. juice of oat grass pulp no. 207; no. 46, control for 4½ weeks, 2.0 gm. dehydrated oat grass no. 208 (Arnold drier); no. 77, control for 5½ weeks, then 10 gamma auxin 'a'; no. 86, 1.0 gm. frozen oat grass pulp no. 207 (batch 2). All received 0.5 cc. orange juice daily.

at this time that it refused to eat the supplement when it was offered. This animal died about 2 weeks later. These results show definitely that the grasses contain a nutritional factor which is essential for maintenance as well as growth of guinea pigs.

The growth curves in figure 4 show that guinea pigs do not need roughage in their diet, at least when milk is used as the

basal ration. One guinea pig received 20 gm. of fresh lawn clippings (mostly Kentucky blue grass) per day as the supplement, while another received 20 cc. of the centrifuged press juice from fresh lawn clippings. Twenty cubic centimeters of this juice contained about 0.2 gm. solids. Both the clippings and the juice were quite effective, although neither was as good as barley grass no. 462, which was fed at a comparable level. Pulped oat grass no. 207 [4] which had been frozen immediately after cutting and pulping was tested and found to be more potent than the freshly cut lawn clippings. Two batches of this pulp were obtained. Of the first batch, 1 gm. was sufficient to promote good growth. The second batch was less potent, 2 gm. per day being required. Since this pulp contained only about 25% solids, the effective levels were 0.25 to 0.50 gm. per day on the dry basis. The centrifuged press juice from this pulp was also effective; 1.5 to 3 cc. per day, containing 8% of solids or 0.12 to 0.24 gm. dry matter, gave definite growth stimulation. A portion of the grass used to prepare pulp no. 207 was dried in an Arnold drier [5] and designated as grass no. 208.[6] This dehydrated oat grass was fed to a guinea pig on the winter milk basal ration, and it was found that about 2.0 gm. daily were necessary to produce good growth. Hence in the dehydration process some destruction occurred.

Since green plant tissues which are growing most rapidly are the most potent sources of the factor in question, it was thought that it might be worthwhile to test the plant hormone, auxin. Hence, auxin 'a' [7] was fed at a level of 10 gamma per day to a guinea pig on the winter milk basal diet. The growth

[4] See footnote 3, page 450.

[5] In the Arnold drier (also called Heil drier and made by the Heil Company), the initial temperature was 1400°F. and the final temperature 250°F. The total time of drying the grasses was 3 minutes. During this period, evaporation proceeds at such a rate that the actual temperature of the grass is considerably less than that of the surrounding gas. Natural gas was used as fuel. The combustion gases are drawn through the drying drum, and give a partial CO_2 atmosphere.

[6] See footnote 3, page 450.

[7] Kindly furnished us by Dr. F. Kögl, Utrecht, Holland.

curve is also given in figure 4. Although no response was obtained, the possibility that auxin is the factor is not excluded since the level fed may have been too low. Our supply was not great enough to feed other pigs at higher levels.

Some idea as to the stability and solubility of the active principle of the grasses was obtained in later experiments, the results of which are given in figure 5. The effect of temperature during storage of grass is shown by the growth of the animals receiving grasses no. 75 and no. 76.[8] These samples were portions of the same batch of dehydrated rye grass, which were stored under different conditions for a period of 6 months. Grass no. 75 was kept in a cooler at a freezing temperature, while grass no. 76 was kept at room temperature which at one time rose as high as 95°F. It is evident that considerable loss occurred during storage at room temperature, while the sample stored in the cold retained its activity.

Lot no. 73,[8] a mixture of wheat and rye grasses, was divided into two parts. One-half of the mixture was fed to a guinea pig as a supplement to mineralized winter milk, while the other half was autoclaved for 1 hour at a pressure of 15 pounds per square inch, dried in the drying room (65°C. for 24 hours), and tested on another guinea pig. The weight records indicate that much of the activity was destroyed by this treatment.

Three samples of dried alfalfa were tested, two of which had been produced under ordinary farm conditions as contrasted to the third, sample no. 380,[8] which had been cut at the stage of most rapid growth and dried in an Arnold drier.[9] The latter sample was quite potent, while the field-dried samples were very poor in growth promoting activity. This difference cannot be ascribed entirely to the method of drying, since the samples were not cut at comparable stages of growth, and further the inactive samples had been stored at ordinary barn temperatures, while sample no. 380 was kept in the

[8] See footnote 3, page 450.
[9] See footnote 5, page 452.

cooler until the feeding tests were made. Also the samples were grown on different soils in different sections of the country.

Other growth curves given in figure 5 show that the factor is not extracted from dehydrated barley grass (no. 462) by

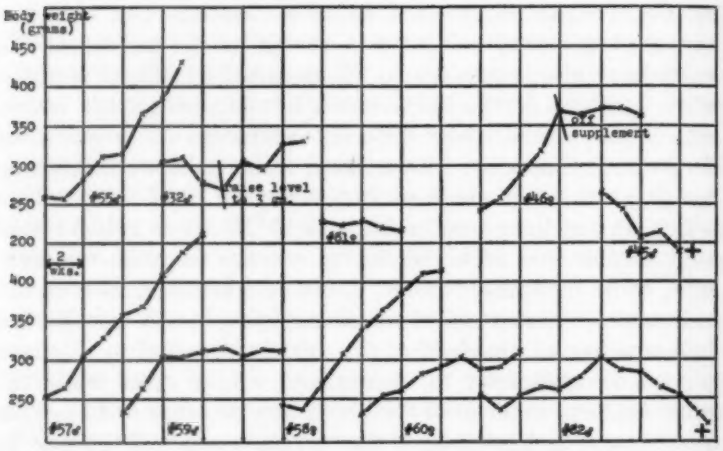

Fig. 5 Weight records of guinea pigs showing the stability of the grass juice factor to heat and its solubility in petroleum ether. The daily supplements were as follows: no. 55, 2.0 gm. dehydrated alfalfa no. 380 (Arnold drier); no. 32, 2.0 gm. alfalfa hay, sample 1; no. 81, 2.0 gm. alfalfa hay, sample 2; no. 46, residue from petroleum ether extraction of grass no. 462; no. 45, petroleum ether extract of grass no. 462; no. 57, 2.0 gm. dehydrated rye grass no. 75 (stored in cooler for 6 months); no. 59, 2.0 gm. dehydrated rye grass no. 76 (stored at room temperature for 6 months); no. 58, 2.0 gm. wheat and rye grass no. 73; no. 60, 2.0 gm. autoclaved grass no. 73; no. 82, control. All received 0.5 cc. orange juice daily.

petroleum ether (Soxhlet extraction for 24 hours). The fractions were fed at levels corresponding to 2 gm. per day of the original grass. The petroleum ether extract, which constituted about 5% of the original grass, was inactive, while the residue retained most of the potency.

DISCUSSION

Although at present there is no indisputable proof that the rat and guinea pig factors are the same, the following considerations indicate that this is the case.

The same basal ration, winter milk supplemented with minerals, has been used to produce the deficiency in both species.

Sources of the potent factor (or factors) are parallel in activity for the two species. Grass samples which were effective for one species have proved to be potent for the other, and, conversely, grass samples which have been inactive for one have also proved to be inactive for the other species. Further, yeast showed inferior activity for both species.

The limited data available at present on loss of activity of grasses upon heat treatment, drying, and storage are parallel for the two species.

The pressed-out juice of potent grasses contains the active factor for both rats and guinea pigs.

Up to the present we have not been able to obtain good growth in guinea pigs receiving mineralized whole milk produced by cows on pasture (Summer, '37). This can probably be attributed to the fact that the pastures were in a very poor condition due to a sustained dry spell. Further, the guinea pig's requirement for the factor is apparently much greater than that of the rat, so that a 'summer milk' which would support good growth in the rat might not be good enough to support growth in the guinea pig. Further work will be done with 'summer milk' when better pastures are available.

In this connection it is interesting that Riddell et al. ('36), in studies designed to show the effects of winter and summer rations of cows on the vitamin C content of the milk, report that, although no appreciable difference was noticeable in incidence of scurvy, 40 cc. of pasture milk fed as a supplement to the basal scorbutic diet produced much better growth in guinea pigs than did a similar supplement of milk produced by cows on a dry ration. Cows receiving silage produced a

milk of intermediate growth promoting qualities. It is probable that these differences were associated with the growth factor with which we have been working.

In view of the indications pointed out above we have assumed, as a working hypothesis, that the rat and guinea pig factors are the same, and further studies are being carried out on stability, chemical properties, and concentration using the latter species for assay.

As was pointed out in our previous paper, the combination of mineralized winter milk, orange juice and yeast supplies in adequate quantities all of the essential minerals, protein and energy, as well as the following known vitamins, C, P, A, B_1, B_6, nicotinic acid amide (anti-blacktongue factor), factor W, flavin, choline and the chick antipellagra factor. Further, unpublished data from this laboratory have shown that several grasses which are rich in the 'grass juice factor,' are very poor sources of the chick pellagra factor, vitamin B_4, the chick gizzard factor, and the highly unsaturated essential fatty acids. That vitamin D was not involved was shown in earlier work by feeding cod liver oil to rats on mineralized winter milk. It is on the basis of the above facts that we postulate the existence in grasses, and less abundantly in other food materials, of a new essential factor in nutrition.

An interesting fact brought out by this work is that guinea pigs can be raised on an all liquid diet in spite of the fact that their digestive tract is equipped to handle large amounts of roughage. Thus, the animals receiving mineralized milk, orange juice and grass juice grew at a good rate and no abnormalities were observed. When the grass juice was omitted the animals died. In contrast to guinea pigs, rats on a mineralized winter milk diet do not die, but grow continuously, although at a slow rate.

Several earlier workers (Bartenstein, '05; Moro, '07) attempted to raise guinea pigs and rabbits on all milk diets, and were uniformly unsuccessful. Their work, however, was done before it was appreciated that milk is low in iron, copper and manganese, so that deficiencies of these elements may have complicated the picture.

Several workers have attempted to raise calves on a milk diet supplemented with iron and copper. Bennet ('32) and Herman ('36) reported that, even with the addition of Fe, Cu, and Mn, calves will not live more than 8 to 13 months on an exclusive milk diet. Herman stated that although the calves grew better than normal on the mineralized milk diet for the first 6 to 8 months, they began to show signs of debility and lost weight after this period. All the animals died by the thirteenth month except one which lived to the seventeenth month. It would be interesting to repeat his experiment adding supplements of a potent grass juice to his ration. By such a method it might be possible to raise a calf on a liquid diet.

Duncan, Huffman and Robinson ('35) attribute the failure of complete nutrition in calves, limited to a milk diet, to a low blood magnesium.

Virtanen ('36) and Virtanen and Lane, ('36 a, '36 b) have published a series of papers showing that various green plant constituents reach maximum concentrations when plant growth is most rapid. Thus, protein, tryptophane, aspartic acid, carotene, and vitamin C are present in the largest quantities just before the plant reaches the flowering stage.

Hunt, Record and Bethke ('36) have shown similar variations in the vitamin B_1 and flavin content of pasture grasses and hays which were correlated with the rate of growth of the plants.

We would like to emphasize the fact that the potency of grasses in the growth stimulating factor with which we have been working also seems to vary in a similar manner with the stage of growth, the mature plants being much less effective than rapidly growing ones. Ordinarily, farmers allow their hay crops to reach a mature stage of growth before harvesting and at this stage the growth-stimulating activity is relatively low. This fact together with destruction of the active principle during drying and storage accounts for the seasonal variation in milk which has been discussed in an earlier paper. This seasonal variation led to the discovery

of the grass juice factor which later work has shown to be distinct from other known nutritional essentials.

SUMMARY

1. Winter milk supplemented with iron, copper, and manganese is an inadequate diet for young guinea pigs. In contrast to rats, which grow slowly on mineralized winter milk, guinea pigs die on such a diet.

2. Orange juice, brewers' yeast, and liver extract produce little or no beneficial effect when fed as supplements to this diet.

3. Various grasses contain a factor (or factors) which is essential for maintenance and growth of guinea pigs. Small supplements of such grasses enable guinea pigs on a mineralized winter milk diet to grow normally.

4. The active principle of grasses is soluble in the plant juices since centrifuged grass press juice is active.

5. The activity of grasses disappears upon storage at room temperature. It is fairly stable at lower temperatures. It is destroyed to a large extent by autoclaving.

6. From experience with the guinea pig it is probable that this species can be used to good advantage in further studies on the 'grass juice factor.'

We are indebted to Mr. S. B. Randle for help in carrying out some of the more recent work reported in this paper.

LITERATURE CITED

ARMENTANO, L., A. BENTSÁTH, T. BÉRES, ST. RUSZNYÁK AND A. SZENT-GYÖRGI 1936 Über den Einfluss von Substanzen der Flavongruppe auf die Permeabilität der apillaren. Vitamin P. Deut. Med. Wochsch., Nr. 33, S. 1325.

BARTENSTEIN, L. 1905 Beitrage zur Frage der Künstlicher Morbus Barlow der Tieren. Jahrb. Kinderheilk., Bd. 61, S. 61.

BENNET, F. 1932 The deficiencies of milk as an exclusive diet, with special reference to mineral constituents. Master's Thesis, Univ. of Minn., St. Paul, Minn., quoted by Herman, 1936.

DUNCAN, C. W., C. F. HUFFMAN AND C. A. ROBINSON 1935 Magnesium studies in calves I. Tetany produced by a ration of milk or milk with various supplements. J. Biol. Chem., vol. 108, p. 35.

ELVEHJEM, C. A., E. B. HART, H. C. JACKSON AND K. G. WECKEL 1934 The nutritonal quality of milks—raw vs. pasturized and summer vs. winter. J. Dairy Sci., vol. 17, p. 763.

HERMAN, H. A. 1936 Growth and development of dairy calves on a milk diet. Mo. Agr. Exp. Sta. Research Bull. 245.

HUNT, C. H., P. R. RECORD AND R. M. BETHKE 1936 The influence of the stage of maturity on the chemical composition and vitamin B(B_1) and G content of hays and pasture grasses. Ohio Agr. Exp. Sta. Bull. 576.

KOHLER, G. O., C. A. ELVEHJEM AND E. B. HART 1936 Growth stimulating properties of grass juice. Science, vol. 83, p. 445.

———— 1937 Further studies on the growth promoting factor associated with summer milk. J. Nutrition, vol. 14, p. 131.

MORO, E. 1907 Experimentelle Beiträge zur Frage der künstlichen Sauglingsernährung. Münch. Med. Woch., Bd. 45, S. 2223.

RIDDELL, W. H., C. H. WHITNAH, J. S. HUGHES AND H. F. LIENHARDT 1936 Influence of the ration on the vitamin C content of the milk. J. Nutriton, vol. 11, p. 47.

VIRTANEN, A. I. 1936 Vitamins and plants. Nature, vol. 137, p. 779.

VIRTANEN, A. I., AND T. LAINE 1936 a Amino acids of plants at different stages of growth. Nature, vol. 137, p. 237.

———— 1936 b Investigations on the amino acids of plants. I. Tryptophane content of leguminous plants at different stages of growth. Biochem. J., vol. 30, p. 1509.

THE TECHNIC OF MEASURING RADIATION AND CONVECTION [1]

JAMES D. HARDY AND EUGENE F. DU BOIS

WITH THE TECHNICAL ASSISTANCE OF G. F. SODERSTROM

Russell Sage Institute of Pathology in affiliation with the New York Hospital and Department of Medicine, Cornell Medical School, New York

ONE FIGURE

(Received for publication November 29, 1937)

Heat is the most important end product of the chemical reactions within the body but relatively little attention has been paid to the mechanism of heat loss. Vaporization which accounts for one-quarter of the loss has been studied in detail. Only a few attempts have been made to separate radiation from convection and the word convection is hardly mentioned in physiological literature. Until fairly recent times the words conduction and Leitung were employed lumping together the two factors to which physicists apply the terms conduction and convection.

The thermal radiation from the human body is normally the largest factor of the heat loss. The amount of energy thus lost may be calculated from the formula of Stefan and Boltzmann. This fundamental equation which contains all the factors upon which the radiation loss depends is:

$Q, k \cdot e \cdot e' \cdot S_o (T_b^4 - T_o^4)$.

Q, number of calories radiated by the surface.

k, proportionality factor depending upon the effective radiating surface area (the profile area according to Lambert's law).

e, emissivity of the receiver (the 'blackness' of the surroundings from the radiation standpoint).

e', emitting power of the emitter ('blackness' of surface of the body).

S_o, Stefan—Boltzmann constant.

T_b, absolute temperature of the emitter (temperature of the surface of the body in °C. + 273).

T_o, absolute temperature of the absorber.

[1] Clinical Calorimetry no. 49.

Convection accounts for about 15% of the heat loss when a man is quiet but for much more than this if there be any considerable movement of air over the surface of the body. The term includes heat removed from the surface by currents of air or water. Attempts to formulate laws for convection have not been attended with any degree of success and most measurements are made by indirect methods. The factors involved under ordinary conditions are:

T_b, the temperature of the surface of the body.
T_a, the temperature of the air.
A, the area of the body.
V_a, the velocity or movement of the air with respect to the body surface.
K, proportionality factor which contains the specific heats of the convecting molecules, diffusion coefficients, etc.

Vaporization which accounts for about 25% of the total heat loss has been studied by many observers and a bibliography of this subject will be found elsewhere (Du Bois, '36). The factors involved are:

T_b, the temperature of the surface of the body.
T_a, the temperature of the air.
H, humidity of the air.
V_a, movement of the air with respect to the moist surface.
A, the moist area of the body.
S, the activity of the sweat glands depending on exercise, room temperature, emotion, etc.
D, the water content of the tissues, a factor of doubtful importance.

Conduction seldom plays an important role unless the body is in contact with a large and highly conducting surface. The amount of heat lost or gained depends on the temperature of the body and of the solid, the area and nature of contact and the specific conductivity of the solid and of the body tissues.

The warming or cooling of ingested food and drink is a factor that can be readily calculated. The loss of heat in urine and feces is taken care of in the calculation for warming the ingesta. The warming of air in the respiratory passages is included under the term convection; the loss of heat through the almost but not complete saturation of air in the nose and air passages is included under vaporization.

REVIEW OF LITERATURE

Rubner (1896) published figures regarding the partition of heat loss from a clothed man under one set of conditions but did not describe his methods, which could not at that time have been accurate. He had a full realization of most of the important factors and he very properly calculated the effective radiating surface as being less than the total surface. However, in his classic book ('02) he does not pay any attention to the division of losses between radiation and convection. Many others devised radiometers which were probably inaccurate. Aldrich made an accurate but rather cumbersome instrument ('22) and published ('28) some valuable data on radiation from the human body. Bohnenkamp ('32) and his co-workers aroused new interest in the subject and emphasized the importance of the effective radiating surface or profile surface. Bedford ('35 a, b) made good use of a radiometer. The most important recent discussion of technics will be found in the papers of Winslow, Herrington and Gagge ('36 a, b).

The respiration calorimeter of the Russell Sage Institute of Pathology (Lusk, '15) which has been used almost continuously since 1914, determines the total heat production by the method of indirect calorimetry. It also measures the total heat loss by the direct or physical method. Vaporization is measured by absorbing the water vapor in bottles of sulfuric acid which are weighed at the end of each period. All the heat of radiation plus convection is caught and measured by the stream of cold water that flows in the pipes near the top of the calorimeter. The accuracy is tested at frequent intervals by alcohol and electric checks. An idea of the operating precision during the last few years may be had from a consideration of the alcohol checks shown in table 1.

Attempts were made to devise a method of measuring convection directly but without success. It therefore became necessary to determine it by difference and this could be accomplished only if radiation were measured with accuracy. There being no satisfactory radiometer available at the time, Hardy ('34) devised one which proved rapid and accurate.

Since the characteristics of radiation vary greatly with wave length it was necessary to make spectrographic studies of the human radiation and its transmission through various media. For this purpose an infra-red spectrograph was devised by Hardy and will be described in another publication. Studies were next made by Hardy and Muschenheim ('36) which confirmed the generally accepted views that radiation from the human skin (temperature 20 to 37°C.) is all given off in the infra-red region with wave lengths between 5 μ to 20 μ with a maximum at about 9 μ. This is far below the range of the sun's radiation since practically none of its waves longer than 2 μ reach the earth's surface. In this region the human skin is within 1 or 2% of being a 'perfect black body radiator.'

TABLE 1

Alcohol checks made during experimental seasons

Date	*Error, per cent* [1]			*Average R.Q.*
	Heat	O_2	CO_2	
February 12, 1935	+ 1.6	— 0.4	+ 0.4	0.661
November 14, 1935	— 0.9	+ 1.4	+ 0.8	0.666
May 15, 1936	+ 1.4	— 0.9	+ 1.8	0.666
February 2, 1937	— 0.2	— 1.0	— 0.8	0.667
March 11, 1937	+ 1.6	— 1.3	— 1.7	0.664

[1] Oxidation of 10 cc. of solution = 52.4 Cals., 14.14 gm. CO_2, 15.42 gm. O, and H_2O, 9.39 gm.

It absorbs practically all of this infra-red radiation, reflects none and transmits none. Layers of epidermis 0.1 mm. in thickness absorb 95% of these radiant heat waves and transmit the resulting heat by conduction and convection. Hardy and Muschenheim ('36) found no significant difference between the white skin and the deeply pigmented Negro skin in this range of wave length which is so far below the visible spectrum. Since white skin is practically a perfect black body, radiation depends directly on the skin temperature and the radiation technic, as shown by Hardy ('34), is the simplest method which will give skin temperatures with accuracy. The Hardy radiometer consists of eight blackened tinfoil receiving elements made up of eight bismuth alloy thermocouples and eight compensating thermocouples. Radiation of heat from the area of 10 sq.cm. at which the instrument 'looks' is concentrated by

means of a silver cone so that it all falls on the blackened disc. Calibration is made by means of a Leslie Cube into one side of which is let a blackened cone which is, for practical purposes, a perfect black body radiator. All calibrations are made at the temperatures of the surfaces to be tested and therefore at indentical wave lengths. Readings which require about 10 seconds can be made on scales which show skin temperature and also radiation rate in small calories per square centimeter per second.

MEASUREMENT OF RADIATION

An exact determination of the total radiation from the human body requires many measurements and calculations. In our calorimeter experiments the naked man lies on a bed made of fish line with a mesh of about 4 cm. Under his hips and back is placed a single cotton sheet folded so as to give the necessary protection to the skin with as small an exposed surface as possible. Between the fish net head rest and the back of the head is a small folded hand towel. Conduction through the fish lines is so small that it can be neglected. The calorimeter which is just large enough to contain bed and man comfortably is a closed circuit apparatus with copper walls, a double plate glass window 75 $\times$ 70 cm. at the head and a small triple glass window 30 $\times$ 30 cm. on the right side. Glass is impermeable to radiation between 3.5 μ and 20 μ. The effective radiation temperature of the copper walls and bottom and the glass window which is measured at the same time as the radiation of the body, is always within one-tenth of a degree of the temperature of the air in the calorimeter. The copper coils at the top of the box are usually 3.5°C. cooler.

The average radiating temperature of the surface of the body is determined by pointing the radiometer at the twenty points on the surface shown in figure 1. These measurements are made by the subject himself just before and just after each basal hour and require only 2 to 3 minutes. The radiation values are read and recorded by an observer who watches the subject through the side window. The total surface of the body is estimated from the Sage height-weight, and linear

formulas (Du Bois and Du Bois, '16). The proportions of surface contributed by head, trunk, arms, legs, hands and feet are calculated from the linear formula and the average radiation per square centimeter from each of these is then multiplied by the percentage of the total area contributed by that part of the body.

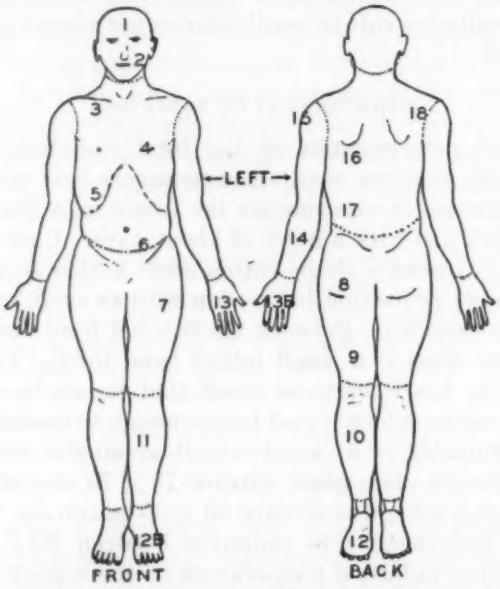

Fig. 1 Locations of individual areas over the body surface which were tested and the divisions of the skin surface for weighting.

From the above data the average skin temperature of the body may be calculated as follows:

$$T_s = (T_1 \times 0.07 + T_2 \times 0.14 + T_3 \times 0.05 + T_4 \times 0.07 + T_5 \times 0.13 + T_6 \times 0.19 + T_7 \times 0.35)$$

where T_s = average skin temperature

T_1 = head temperature	0.07 = weight for head surface
T_2 = arms temperature	0.14 = weight for arms surface
T_3 = hands temperature	0.05 = weight for hands surface
T_4 = feet temperature	0.07 = weight for foot surface
T_5 = legs temperature	0.13 = weight for leg surface
T_6 = thighs temperature	0.19 = weight for thighs surface
T_7 = trunk temperature	0.35 = weight for trunk surface

The various weighting factors were computed from the linear formula measurements of sixteen subjects.

The radiation temperature of the internal calorimeter surfaces is measured so that the radiation exchange between the subject and the calorimeter may be made. Thus

$$Q = 1.37 \times 10^{-11} (T_s^4 - T_c^4) \times t \times A \times f$$

Q = kg. Cals. per period lost to the calorimeter from the skin
T_s = average skin absolute temperature
T_c = average calorimeter absolute temperature
t = length of the period in seconds
A = total area in square meters
f = ratio of the effective radiating area to the total area = 0.78

The relationship of effective or profile radiating surface to the total surface area depends on the position of the body and extremities. We have adopted the standard position of lying flat on the back, legs close together and arms close to the body. Bohnenkamp and Ernst ('31) using a spread eagle position estimated the profile surface as 85% of the surface according to the linear formula. Bandow and Bohnenkamp ('35) using the electrical capacity method in improved form find an average of 81% for men and 86% for women.

Bedford ('35) who studied men in the crouching position estimated 50%. Winslow, Herrington and Gagge, who made observations on two men, seated in a chair, obtained percentages of 74 and 75, values lower than those of Bohnenkamp as might be expected from the differences in position. We have made measurements on two normal men, one E.F.D.B., the subject of many experiments, and the other V.T., a man with short legs but exactly the same sized trunk as E.F.D.B. They were placed in the position of Egyptian mummies and wrapped in gummed paper according to the method of Du Bois and Du Bois ('16). Their 'mummy' surfaces were 78.3 and 78.4%, respectively, of their total surfaces. For purposes of calculation we have adopted for the present the standard figure of 78%, realizing that there may be an error as great as 2 to 3%.

The radiation exchange may be precisely determined by measuring the projection area of each part of the body surface and the radiation exchange of this surface with the part of the environment to which it is exposed. These values are integrated over a hemisphere and then added. Such a procedure is described in detail by Bohnenkamp and Pasquay ('31), although their numerical values are not correct. Such an experiment was performed on E. F. D. B. The radiation loss, using the photographically determined effective area, was 46.8 Cals. per hour and that measured by the weighting method just described was 45.8 Cals. per hour. The 2% difference is considered to be within the estimated experimental error of $\pm$ 3%.

CONVECTION

The stream of cold water which flows through the cooling pipes near the roof of the calorimeter removes all the heat of convection and radiation together. The temperature of the water is measured as it enters and as it leaves the box and the water that has flowed through the box is weighed at the end of each period When the heat of radiation is subtracted from the calories found by this direct determination it leaves the heat lost by convection. The factor of conduction through the netting is too small to be significant.

In our experiments there was no ingestion of food or drink. When urine was voided into a wide necked bottle it was weighed on a Chatillon balance inside the calorimeter and its heat loss measured by thermometry or calculated from known rates of heat loss determined in many experiments. From a thermal standpoint the urine is considered as a separate body and its heat loss subtracted from the total to find the heat loss of the human subject.

The air flow through the calorimeter is adequate to remove the body gases but not sufficient to affect heat loss. The air enters the head of the calorimeter and is baffled so that there is no movement of air perceptible to the naked subject or to a delicate anemometer. The average humidity inside the box

is determined from the water 'residuals' (the water vapor content of 10 liters of calorimeter air which is tested at the end of each period). The humidity is usually low, ranging from 22% to 47%, the ordinary level being about 30%.

VAPORIZATION

The current of air leaving the calorimeter is passed through a blower and then through two tall glass flasks which contain concentrated sulfuric acid. These are weighed at the end of each period giving the water vaporized after corrections are made for changes in the moisture content of the air as determined by gravimetric analysis of 10 L samples of air at the end of each period. Care is taken to adjust the ventilation of the box so that there is little or no change in the humidity. This reduces to a minimum the error caused by loss or gain of water by the copper walls, bed or sheet. No experiment is started until the subject has been lying on the sheet at least an hour and sealed in the calorimeter ¾ hour. No experiments are attempted on days when the external humidity is high, as the walls on such days lose several grams of water in the early part of the experiment.

In calculating the heat lost in vaporization we employ the standard figure of 0.583 Calories for each gram of water which evaporates at the average calorimeter temperature of 25°C. It may be pointed out that to expand the saturated vapor to 25% relative humidity and 25°C. (room conditions) requires an additional 0.062 Calorie per gram which may be considered as part of the vaporization loss as it depends essentially on the number of grams of moisture evaporated. In this paper we have left this small fraction which amounts to 2 to 3% of the total heat loss in the Calories ascribed to convection. Perhaps it deserves a separate heading.

STORAGE OF HEAT

In some experimental procedures such as that described by Winslow, Herrington and Gagge ('36 a, b) the storage of heat in the human body is an important factor in the calculations.

In our procedure it is measured directly by means of the difference between the heat production and the heat eliminated. In addition the temperature of the body is estimated with an electrical resistance thermometer inserted 15 cm. in the rectum and read to 0.01°C. every 4 minutes, and by the twenty surface temperature readings taken at the beginning and the end of every period. Even with all these precautions the measurement is inexact because of differences in the rate of temperature change in various organs of the body and differences in the specific heats of these organs. This problem has been studied by the Sage investigators for the last 24 years. Coleman and Du Bois ('15) found evidence that with rapid changes of body temperature thermometers strapped on the surface gave better indications of average body change than rectal thermometers. Barr and Du Bois ('18) devised a method of determining average body change by direct calorimetry from the total heat produced as measured by indirect calorimetry. Burton ('35) using a similar calculation has found best agreement when the rectal temperature change is weighted as 65% and the average surface temperature as 35%. In our preliminary calculations of our own basal experiments we obtain best agreement by weighting the rectal temperature as 80% and the average skin temperature as 20%. This does not hold except in basal or near basal conditions. Gagge ('36) working with normal men finds that the lowering rectal temperature changes alone may be incorrect as indices of storage when the change is large but that rising rectal temperature changes associated with warm conditions ordinarily yield correct results. This, however, is certainly not the case in chills and fever or exercise as shown in a long series of fever studies made in the Sage calorimeter.

Fortunately our calculations of convection and radiation do not involve consideration of this uncertain factor of heat storage in the body. Our basis of calculation is total heat loss from the surface of the body and this is determined directly by physical methods, direct calorimetry. Of course simultaneous measurements of total heat producion are made by the

method of indirect calorimetry using O_2 and CO_2. The storage of heat in or loss of heat from the body is calculated also and an estimation of heat loss is furnished from the algebraic sum of total heat production and heat storage. In large series of experiments the totals of direct and indirect calorimetry agree closely. With individual periods of 1 hour the divergence in normal people may be $\pm$ 8%.

EXPERIMENTAL ROUTINE

The day before an experiment the room thermostat is adjusted to the desired temperature. The temperature of the room can be held to $\pm$ 0.5°C. and the periodicity of the fluctuations is about $\frac{1}{2}$ hour. The calorimeter room is provided with both cooling and heating units so that any desired temperature may be easily obtained. At 8 A.M. of the morning of an experiment the water is started circulating through the cooling coils of the calorimeter and the temperature of the box is maintained by a heating unit in the calorimeter. In this way thermal equilibrium in all parts of the mechanism is well established before the starting of the experimental period. The temperature of the calorimeter is measured at frequent intervals and the heating current adjusted to keep the box temperature about equal to its initial temperature, i.e., before starting the flow of water. Other routine such as rechecking the radiometer calibration and checking the weight of the oxygen tank is completed by the time the subject arrives at about 9 A.M.

The subject who has had no breakfast sits in ordinary indoor clothing for 1 hour in the prevailing atmosphere. At 10 A.M. the subject undresses and at the same time measures the temperature of the skin under his clothing. He is weighed immediately after voiding, the rectal thermometer inserted, and is sealed in the calorimeter at about 10.15. The air circulation is started and the preliminary period is begun. It requires about 30 minutes to allow all the parts of the calorimeter to come again into thermal equilibrium. At 10.45 the subject makes his first measurement of surface temperature and box radiation temperature. The radiometer is pointed

at all parts of the inner surface of the calorimeter, and the readings are weighted according to the surface area of the calorimeter part. The side walls are usually at the same radiation temperature, but the top of the calorimeter is about 0.5°C. cooler than the rest of the box. It has been found that the weighted average of all readings is within 0.1°C. of the average air temperature which is measured by six resistance thermometers located in various parts of the box.

At about 11 A.M. the first experimental period is begun, and during this basal period the subject remains as quiet and motionless as possible. Immediately after the start of the second period the skin and box wall temperatures are again measured. The second and third periods are quite often used for studying the effects of exercise, forced air currents, or chilling, so that the surface temperature may be measured several times during these periods. At the end of the experiment the skin and wall temperatures are again measured. From the data thus obtained all the factors of heat production, heat elimination, and the partition of the latter are obtained, as well as a record of the effect of certain external factors on the skin temperature.

The following is a sample of the calculation of the heat produced and heat eliminated, and the factors of radiation, convection and vaporization.

Date: March 13, 1935
Subject: E.F.D.B., basal experiment, nude
Time: 11.15 A.M. to 12.15 P.M.
Age 52, weight 74.74, height 178 cm.
Total surface area = 1.96 sq.m.
Effective radiating surface area = 1.54 sq.m.
Average calorimeter temperature = 27.40°C.
Average calorimeter humidity = 25%

Heat produced

	Absorbed grams	*Correction for residual air*	*Consumed or produced grams*	*R.Q.*	*Heat produced*
CO_2	25.02	+ 1.11	23.16		
O_2	21.81	— 1.14	20.67	0.815	68.65
H_2O	35.95	— 0.53	35.42		

Heat eliminated

Weight of water flowing through calorimeter = 21.41 kg.
Average temperature of ingoing water = 20.81°C.
Average temperature of outgoing water = 23.48°C.
Change in box temperature (thermal capacity = 16 kg. per degree) = 0.03° drop
Total heat absorbed = 57.16 —0.48 = 56.68 Calories
Heat lost by vaporization = 20.68 Calories
Total heat eliminated = 77.36 Calories

Body heat storage

	Start	*End*
Rectal	37.25	37.10
Average skin	33.02	32.81

Heat storage observed = 77.36 — 68.65 = — 8.71 Cals.
Heat storage calculated from rectal alone = — 9.54 Cals.
Heat storage calculated from 0.8 R + 0.2 S = — 10.32 Cals.

Partition of heat eliminated

Skin temperature

	Start		*Finish*		*Radiation average*
	Temp.	Temp. weighted	Temp.	Temp. weighted	*Cals. per sq.m. per hour*
Head	35.0	245	35.0	245	
Arms	33.3	466	32.7	458	
Hands	34.1	171	33.8	169	
Feet	30.6	214	30.4	213	
Legs	31.8	413	31.5	409	
Thighs	31.9	606	31.6	600	
Trunk	33.9	1187	33.9	1187	
Average skin temperature		33.02		32.91	29.7

Total heat lost by radiation = 29.7 × 1.54 = 45.70 Cals. = 58%
Total heat lost by convection = 56.68 — 45.7 = 11.00 Cals. = 15%
Total heat lost by vaporization = 20.66 Cals. = 27%
Total = 77.36 Cals. = 100%

SUMMARY

The total radiation from the surface of the human body can be determined by means of the Hardy radiometer making due allowance for the effective radiating surface which is smaller than the total surface. The respiration calorimeter of the Russell Sage Institute of Pathology in one set of measurements determines the heat lost in vaporization. By an independent method it gives accurate figures for the heat lost in

radiation plus convection. This makes it possible to determine by subtraction the heat loss by convection and to partition the three important channels of heat loss. Inasmuch as calculations are based on heat loss and not heat production the heat storage in the body is not employed in determining partition of heat loss. It can be calculated from the difference in heat production and heat loss.

It is believed that the combination of respiration calorimeter and radiometer has provided the first accurate measurement of normal convection loss.

LITERATURE CITED

ALDRICH, L. B. 1922 The Melikeron—An approximately black-body pyranometer. Smithsonian Misc. Collections, vol. 72, no. 13.

———— 1928 A study of body radiation. Smithsonian Misc. Collections, vol. 81, no. 6.

BANDOW, F., AND H. BOHNENKAMP 1935 Über die Bestimmung der Strahlungsfläche des Menschen aus seiner elektrischen Kapazität. Arch. f. d. ges. Physiol., Bd. 236, S. 427.

BARR, D. P., AND E. F. DU BOIS 1918 Clinical Calorimetry 28, The metabolism in malarial fever. Arch. Int. Med., vol. 21, p. 627.

BEDFORD, T. 1935 a Skin temperature in relation to the warmth of the environment. J. Hygiene, vol. 35, p. 307.

———— 1935 b The effective radiating surface of the human body. J. Hygiene, vol. 35, p. 303.

BOHNENKAMP, H., AND W. PASQUAY 1931 Ein Neuer Weg zur Bestimmung der für die Wärmstrahlung Massgebenden Oberfläche des Menschen. Pflüger's Arch., Bd. 228, S. 79.

BOHNENKAMP, H., AND H. W. ERNST 1931 Untersuchung zu den Grundlage des Energie und Stoffwechsels. II. Über die Strahlungsverluste des Menschen. Arch. f. d. ges. Physiol., Bd. 228, S. 63.

BURTON, A. C. 1935 The average temperature of the tissues of the body. J. Nutrition, vol. 9, p. 261.

COLEMAN, W., AND E. F. DU BOIS 1915 Clinical Calorimetry 7, Calorimetric observations on metabolism of typhoid with and without food. Arch. Int. Med., vol. 25, p. 887.

DU BOIS, E. F. 1936 Basal Metabolism in Health and Disease, 3rd ed. Lea and Febiger, Philadelphia.

DU BOIS, D., AND E. F. DU BOIS 1916 Clinical Calorimetry 10, A formula to estimate approximate surface area if height and weight be known. Arch. Int. Med., vol. 17, p. 863.

GAGGE, A. P. 1936 The linearity criterion as applied to partitional calorimetry. Am. J. Physiol., vol. 116, p. 656.

HARDY, J. D. 1934 The radiation of heat from the human body. I. An instrument for measuring the radiation and surface temperature of the skin. J. Clin. Invest., vol. 13, p. 593.

HARDY, J. D., AND C. MUSCHENHEIM 1936 Radiation of heat from the human body. V. Transmission of infra-red radiation through skin. J. Clin. Invest., vol. 15, p. 1.

LUSK, G. 1915 Clinical Calorimetry 1, A respiration calorimeter for the study of disease. Arch. Int. Med., vol. 15, p. 793.

WINSLOW, C.-E. A., L. P. HERRINGTON AND A. P. GAGGE 1936 a A new method of partitional calorimetry. Am. J. Physiol., vol. 116, p. 641.

———— 1936 b Determination of radiation and convection exchanges by partitional calorimetry. Am. J. Physiol., vol. 116, p. 669.

RUBNER, M. 1896 Zur Bilanz unserer Wärmeökonomie. Arch. für Hyg., Bd. 27, S. 69.

———— 1902 Die Gesetze des Energieverbrauchs bei der Ernährung. Franz Deuticke, Leipzig.

BASAL METABOLISM, RADIATION, CONVECTION AND VAPORIZATION AT TEMPERATURES OF 22 to 35°C. [1]

JAMES D. HARDY AND EUGENE F. Du BOIS

WITH THE TECHNICAL ASSISTANCE OF G. F. SODERSTROM

Russell Sage Institute of Pathology in affiliation with the New York Hospital and Department of Medicine, Cornell Medical School, New York

SIX FIGURES

(Received for publication November 29, 1937)

There are few studies in the literature dealing with attempts to separate radiation from convection. The first important work was done by Aldrich ('28), and later Bohnenkamp and Ernst ('31) and his associates stimulated research in this field. Burton and Bazett ('36) and Burton ('34) have made valuable contributions. Recently Winslow, Herrington and Gagge ('36, '37 a, b) using the Hardy radiometer have published some important results. Our colleagues, the heating and ventilating engineers, have for several years realized the necessity for this differentiation and a new method of heating rooms based on low temperature radiation has been introduced into England by Barker ('32). The studies of Houghten, Teague, Miller and Yant ('35); and Yaglou ('26), have been of great practical service though their results cannot be compared with ours since they did not measure heat loss and their subjects were clothed and moderately active. There have been many reports on the effect of exposing animals and clothed and naked persons to various temperatures. The literature is summarized by Lusk ('28), Deighton ('33), Du Bois ('36), and by Swift ('32), who gives an excellent discussion of shivering.

[1] Clinical Calorimetry no. 50.

The physical factors in body temperature maintenance and heat elimination are well reviewed by Deighton.

Our experimental technic has been described in the previous paper (Hardy and Du Bois, '38). Only two normal subjects have been fully studied as it seemed wiser to make many experiments on two people rather than few observations on many individuals. It has required almost a year's work of four experimenters to complete the observation on one subject.

EXPERIMENTAL SUBJECTS

E.F.D.B., physician, born June, 1882, age 54, height 179 cm., weight 77.5 kg., average build, has been the subject of many respiration experiments since 1909 (Du Bois, '36). In most of these he lay lightly clothed in a calorimeter at comfortable air temperature. His metabolism has been unusually constant, falling slightly with advancing age. His weight has not varied significantly and his mode of life has been uniform. He exercises regularly, running about 1¼ miles every morning before breakfast except, of course, on experimental days. During the last 5 or 6 years there has been a slight irregularity of the heart due to nodal rhythm, a condition not infrequent in persons with slow heart rates. This irregularity disappears in exercise and in no way affects the circulation. X-rays of the extremities show the slight calcification of the vessels consistent with his age. He has lived almost all his life in New York and Massachusetts and stands cold air and cold water as well as the average man. His response to heat by sweating is about average. In the experiments made by C.G. and F.G. Benedict and Du Bois ('25) he was exposed to very dry, very hot air entering an oil cloth bag at a temperature of 94°C. His metabolism was increased only 7% in spite of the fact that his feet and legs were almost unbearably hot.

J. D. H., physicist, born in Texas, 1904, age 33, height 168 cm., weight 67 kg., is of a shorter, stockier build than D, with a little more subcutaneous fat. He has always been athletic and he plays a hard game of squash about three times a week.

His health is good except for occasional colds. Physical examination shows normal heart and lungs. Until the age of 23 he lived in Mississippi, since then has been in Maryland, Michigan and New York. He is about as well adjusted to cold as the average New Yorker. His sweating response to warm environments or to exercise is about average.

EXPERIMENTAL CONDITIONS

The experiments were started in the neutral zone of temperatures, 27°C. to 29°C., and then extended to the extremes of heat and cold with repetitions and interruptions so that the final curve would not be affected by any possible seasonal variation if such exists. Experiments were made during the spring and winter months of 1935, 1936 and 1937. The subjects came to the laboratory having had no breakfast except perhaps a cup of caffeine free coffee or a cup of hot tomato juice. In the winter they were normally dressed for the trip to the hospital. Arriving at about 9 A.M., they sat dressed in ordinary clothing until about 10 A.M. when they undressed. As their clothes were removed measurements were made of skin temperature. About 10 A.M. they were placed in the calorimeter and about 11 o'clock the experiment began. Tests on these two men and a number of others have shown that at the lower range of temperatures the skin cooled rapidly for the first 90 minutes and then fell much more slowly. In the warmer air range the fall of skin temperature was much less marked and uniformity was rapidly attained. In the cooler experiment the subjects were cold and slightly uncomfortable during the preliminary period and first basal hour. Eventually the combination of cold air and absolute quiet brought about distinct chilly sensations with an involuntary tensing of muscles which involved no visible shaking. This gradually increased over a period of 5 to 20 minutes before there was a violent chill with chattering of the teeth and clonic movements of the extremities which shook the whole box. It is interesting that both men maintained their metabolism at the ordinary basal levels until a few minutes before the chill began and

they were able to warn the observers about 10 to 15 minutes before the approaching chill so that a second experimental period could be started which would include the onset of shivering. At the highest temperatures the subjects were sweating from the beginning of the preliminary period to the end of the experiment. At a certain critical warm temperature there would be a constant sweating in the axillae with transient outbreaks of sweat over the body when the subject expended the extra two or three calories involved in making the surface temperature readings. Except for these measurements and for occasional turnings from side to back the men lay almost motionless during the basal hours. Sometimes they dozed lightly for a few minutes.

After a satisfactory basal hour had been obtained the second and third periods were used for a study of the effects of exercise or of an electric fan. The data of all the basal experiments on the nude subjects are given in table 1; other basal periods, associated with exercise and chill experiments, will be found in subsequent papers. The experiments are arranged in order of increasing calorimeter temperature, and the first part of the table is concerned with gaseous exchanges involved in calculation of heat production. The later columns contain the thermal data and the heat eliminated. In this report all the nude basal periods are considered together.

DISCUSSION

Skin, rectal and average body temperatures

Skin temperature. The rectal and surface temperatures of the various parts of the body at different calorimeter temperatures are shown in figures 1 and 2. Each vertical set of readings was made on a different day but the results are surprisingly consistent. It happened in both subjects that the line for the temperature of the hands ran very close to the weighted average of the whole surface. If this should be the case for a large number of persons it would be a point of considerable practical value. In warm atmospheres the extremities and other parts of the skin surface have temperatures ranging within $\pm 1°$ of the surface of the trunk.

TABLE 1

Two-hour basal experiments on subjects H and D

SUBJECT, DATE, WEIGHT, REMARKS	END OF PERIOD	CO_2	O_2	R.Q.	H_2O	URINARY N PER HOUR	PULSE RATE	TOTAL HEAT PRODUCED	CALORIMETER TEMPERATURE	CALORIMETER HUMIDITY	RECTAL TEMPERATURE	TOTAL HEAT ELIMINATED	SKIN TEMPERATURE		RADIATION	CONVECTION	VAPORIZATION
													Time	Temperature			
		gm.	*gm.*		*gm.*	*gm.*		*Cal.*		%		*Cal.*			%	%	%
JDH. Feb. 18, 1937, 66.7 kg.	11:08										36.78		10:48	32.9			
1st period basal; 2nd	12:08	18.9	16.9	0.81	24.5	0.51	53	56.0	25.40	23	36.63	74.2	12:20	31.9	70	11	19
period quiet; cold at end	1:08	23.4	20.4	0.83	26.1		49	68.2	25.38	22	36.66	77.0	1:20	31.6	61	19	20
JDH. Feb. 4, 1937, 67.2 kg.																	
Pajamas and 2 pair socks	10:58										37.08						
Both periods basal. No	11:58	21.2	17.8	0.87	25.9		59	59.9	26.53	25	37.01	61.9					
surface measurements	12:58	21.3	18.3	0.85	25.2	0.42	54	61.5	26.54	25	37.04	61.4					
JDH. Feb. 16, 1937, 66.9 kg.	11:23										36.72		11:00	33.5			
Both periods basal	12:23	24.0	20.1	0.87	27.2		52	67.5	26.91	22	36.63	68.1	12:40	32.9	67	10	23
	1:23	16.1	15.9	0.74	26.4	0.70	51	51.3	26.80	20	36.63	66.2	1:35	32.8	67	10	23
JDH. Feb. 11, 1937, 66.8 kg.	11:09										36.76		10.49	33.6			
1st period basal. 2nd	12:09	19.4	17.4	0.81	27.3		54	57.5	27.67	23	36.71	69.3	12:25	33.0	62	15	23
period basal	1:09	22.3	18.9	0.86	28.6	0.51	54	63.4	27.61	22	36.76	68.0	1:20	32.8	58	17	25
EFDB. Feb. 6, 1936, 77.6 kg.	10:42										37.14		10:20	32.7			
1st period basal. 2nd	11:42	23.8	20.2	0.86	37.9		57	67.9	27.71	28	36.95	83.4	11:45	32.8	53	20	27
period basal	12:42	24.3	21.3	0.83	40.3	0.56	52	70.9	27.64	27	36.88	83.6	12:44	32.6	52	20	28
JDH. Feb. 9, 1937, 66.7 kg.	10:55												10:30	34.1			
1st period basal. 2nd	11:55	19.4	17.8	0.79	32.6		60	58.6	28.58	25		65.8	12:10	33.2	58	13	29
period quiet	12:55	23.6	20.5	0.84	33.8	0.55	60	68.4	28.60	23		66.7	1:05	33.7	55	15	30

In all experiments of this group the head had the highest skin temperature. The extremities, especially the feet, began to show a precipitate fall in temperature when the calorimeter air was lower than 28°C. Also at about this point the subjects

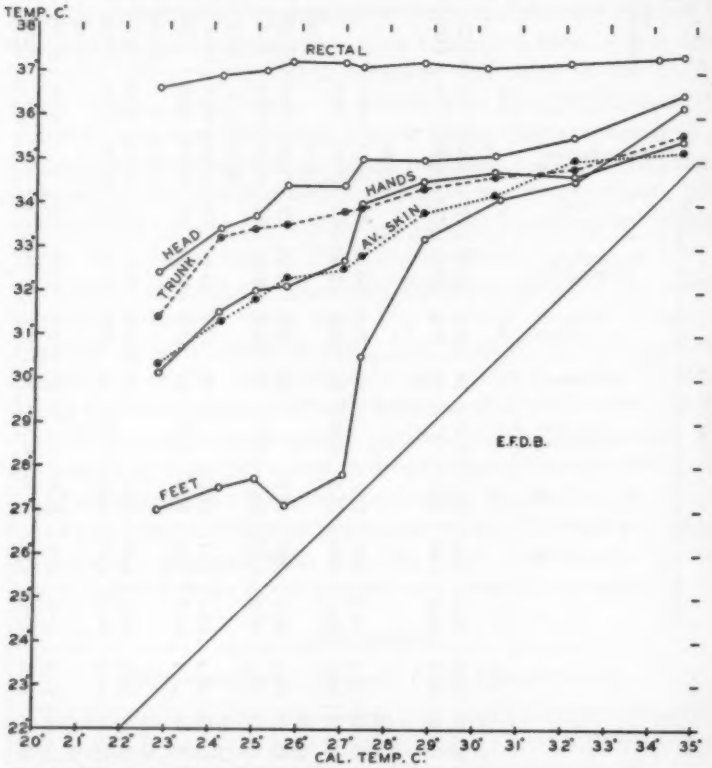

Fig. 1 Skin and rectal temperatures of subject D, during basal experiments at different environmental temperatures.

began to feel cool. In cold environments the temperature of the feet was low, often the surface of the toes was colder than the air on account of the large amount of surface for vaporization, the low heat production of the bones, joints and tendons, poor blood supply and the long distance from the

'central heating apparatus.' The other end of the body, the head, was relatively little affected by the cold air. It has a better blood supply and is accustomed to nakedness in cold weather. The trunk and hands occupy intermediate positions. Although in warm environments the skin temperature rapidly establishes an equilibrium level, upon exposure to cold (Cal.

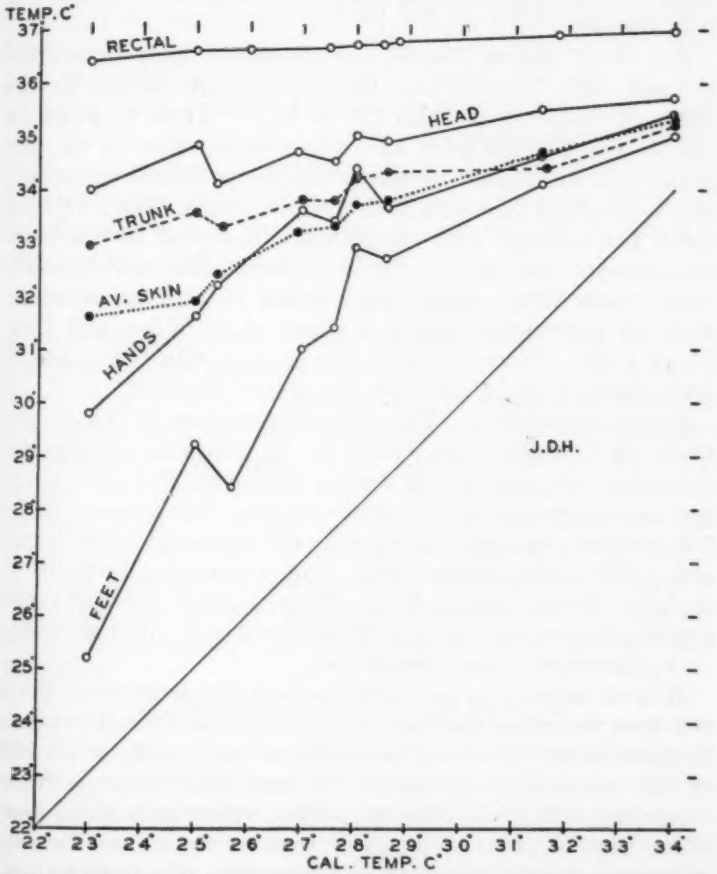

Fig. 2 Skin and rectal temperatures of subject H, at different environmental temperatures.

Temp. less than 26°C.) the skin temperature falls until the subject shivers. It is difficult to speak of a 'level' of skin temperature under these conditions, and the values of skin temperature given for these experiments are the average readings for the beginning and end of the first basal hour. The tendency of all parts of the skin surface to assume the same temperature in warm environments is demonstrated in both subjects.

For every degree rise in environmental temperature the average skin temperature increases about 0.5°C., in the range of temperature from 22° to 29°C. At 29°C. sweating has increased to the point where its cooling effect on the skin is apparent and higher environmental temperatures cause only relatively slight increases in skin temperature. The maximum value for average skin temperature observed in the basal experiments was 35.3°C. It is probable that environments hotter than 36°C. would cause a fall in skin temperature. Such an observation has been reported by Wiley and Newburgh ('31). Figure 6 shows the average skin temperatures for subjects D and H and they are almost identical.

Rectal temperature. The rectal temperature of D averaged about 0.4°C. higher than that of H. In nearly all experiments the rectal temperature fell during the basal, first period, and the fall was greater in cold environments. The average rectal temperature rose with increased environmental temperature and at 23°C. the resting rectal temperature was from 0.6°C. to 0.8°C. lower than at 34°C. Thus, a lower level of rectal temperature of this extent, of itself, will not cause shivering or an increase in heat production.

It is of interest to note that the skin temperature of these two men was about halfway between internal (rectal) and air temperatures. The distances between the lines show the size of the temperature gradients but not their shapes. Some observers, using electrical resistance wires in needles, have found that in parts of the body such as the wrists the subcutaneous temperature may be lower than skin temperature, owing to the cooling effect of the blood in the veins.

Average body temperature. This quantity is of considerable importance from both a laboratory and clinical standpoint. Its importance in calorimetric work is obvious, and from the clinical side the estimation of the average body temperature of patients being treated with hyperpyrexia is very desirable. So far, our observations concern only the resting basal state, and can under these circumstances throw light only on the questions: how well does the rectal or skin temperature represent the average body temperature, and how well does any combination of rectal and average skin temperature represent average body temperature? Barr and Du Bois ('18) made estimates of the changes in average body temperature by subtracting the heat produced in the body from the heat eliminated. Burton ('35) has correlated this difference with the changes in skin and rectal temperature and he arrived at the formula:

$$\text{Average body temperature} = 0.65 \text{ rectal} + 0.35 \text{ skin}$$

He, at the same time, made the observation that the skin temperature was the more important factor even though it is weighted only 35%. Burton's skin thermometer was of a type which would indicate changes rather than the actual skin temperature. The data of the present experiments were studied to determine the relationship between the thermal changes in the rectal cavity, the skin, and the average temperature of the body. It was at once clear that the change in rectal temperature alone would give a fair approximation of the change in average body temperature, although a slightly better approximation could be made using the following formula:

$$\text{Average body temperature} = 0.8 \text{ rectal} + 0.2 \text{ skin}$$

Even after decreasing the weight of the skin temperature term, this factor is still an important one. Du Bois has figured that for a man weighing 70 kg., about 15 kg. is within 1 cm. of the surface. Our equation would lead one to conclude that 14 kg. of the man's mass is to be weighted with the skin temperature.

The simplest way to study the average temperature of the body is to make direct observation of the actual temperature changes by measuring the heat production and elimination. Then

$$-\Delta T_b = \frac{H_e - H_p}{M \times S}$$

ΔT_b = change in average body temperature
H_e = heat eliminated
H_p = heat produced
M = mass of subject
S = average specific heat of body tissues

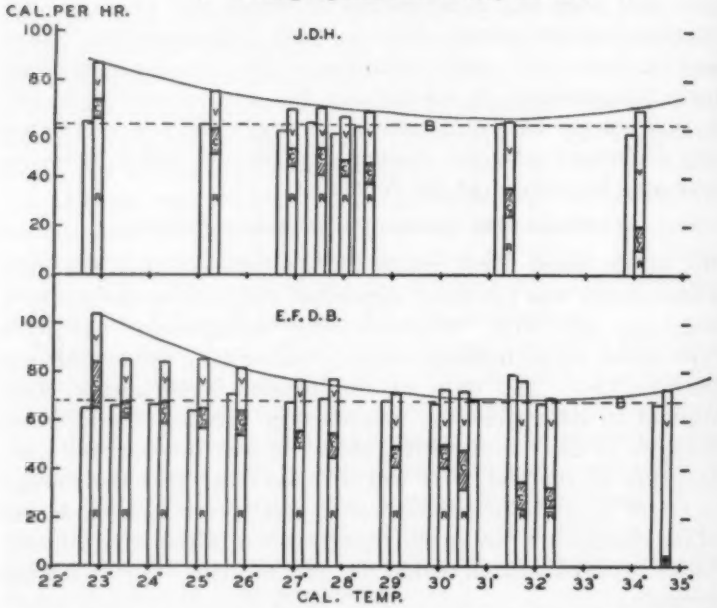

Fig. 3 Changes in heat production and heat elimination for both subjects during the basal periods with calorimeter temperature. Blank columns on left, heat produced; columns on right, heat eliminated; V, heat loss by vaporization; C, heat loss by convection; R, heat loss by radiation.

A value of 0.83 is usually assumed for the average specific heat of the body tissues, and all the other factors except T_b are measured so that this quantity is determined. Correlation of this factor with the temperature measurements on the body can then be attempted. The only bodily temperature

measurements which were made in these experiments were the rectal and average skin temperature. In figure 4 are plotted four sets of combinations of skin and rectal temperature against T_b. The formulae so tested are:

1. $-\triangle T_b = -k\triangle R$
2. $= -k \quad (0.8\triangle R + 0.2\triangle S)$
3. $= -k \quad (0.65\triangle R + 0.35\triangle S)$
4. $= -k\triangle S$

$k = 0.83 \times M$
R = change in rectal temperature
S = change in skin

The change in body temperature was negative in all these experiments; that is, the body temperature was falling. In order to plot the data in the usual way all the signs were reversed.

The relation of rectal temperature change to average body temperature change is seen to be fairly close in these experiments, although the average line does not pass through the origin but about 0.09°C. above the origin. The spread of the values is $\pm$ 0.15°C. By accounting also for the skin temperature with a weight of $0.8\triangle R$ and $0.2\triangle S$, the average curve is above the origin by 0.06°C. and the spread $\pm$ 0.09°C. Using the formula $0.65\triangle R$ and $0.35\triangle S$, the average curve passes 0.05°C. above the origin and the spread is $\pm$ 0.17°C. The skin temperature relation shows a much smaller slope and passes 0.09°C. above the origin with a $\pm$ 0.17°C. spread.

The slope of the average $\triangle R$ curve is greater than unity indicating that the body temperature changes more than is shown by the rectal alone. The slope of Burton's formula is less than unity, evidence of too great a weight on the skin temperature. The slope of the $0.8\triangle R + 0.2\triangle S$ is unity and as the spreading of the data is minimal, this formula is taken to best fit these observations. The slope of the skin temperature curve is very much smaller than unity (only 0.25) showing that the body average temperature does not change anything like as much as the skin temperature. The correlation however between the changes in average skin and average body temperature is good enough to show the importance of this factor.

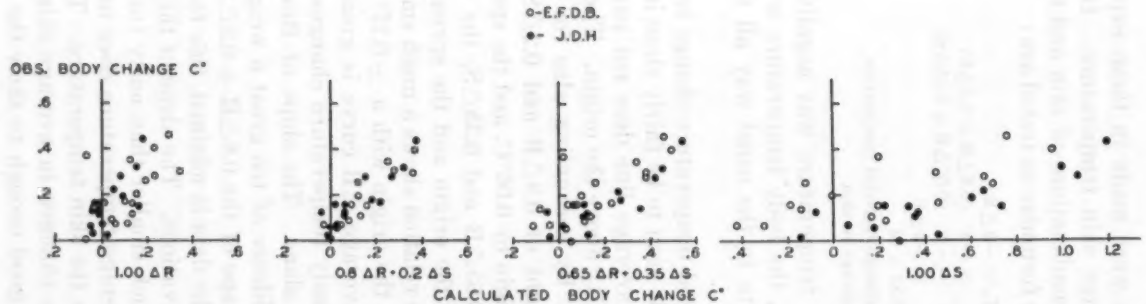

Fig. 4 Relation of observed change in average body temperature to calculated temperature changes from ΔR, changes in rectal temperature, and ΔS, changes in skin temperature.

The fact that none of the curves pass through the origin may possibly be due to a systematic difference between the heat eliminated and heat produced of 2.5 calories, but is more probably due to a slight fall of temperature (0.06°C.) of the rectal cavity associated with muscular relaxation during the first basal period. As will be apparent later, the strict application of any single formula for average body temperature may lead to error, especially during exercise when the skin temperature may fall several degrees in spite of increased body temperature and vaso-dilatation. Patients in chills and fever sometimes show higher skin temperature than rectal temperature, in which case the average body temperature should be related more to the skin temperature. So far as we have studied the matter, no one formula can be used under all conditions.

Average specific heat of body tissue

Assuming that the formula proposed for average change in body temperature represents this value fairly closely, an estimation of the specific heat of the body may be made. Evidently,

$$\Delta C = M \times S \times \Delta T_b = H_p - H_e,$$

where ΔC = heat lost from the body
M = mass of the body
S = average specific heat of body
ΔT_b = average change in body temperature
H_p = heat produced
H_e = heat eliminated

Then, by putting in the formula for average body temperature change,

$$S = \frac{H_p - H_e}{M\,(0.8\Delta R + 0.2\Delta S)}$$

Graphical estimation of S may be had by plotting $H_p - H_e$ against $M(0.8\Delta R + 0.2\Delta S)$, as is done in figure 5.

The slope of the best straight line through these points is the desired value of S, and the extreme values come out 0.83 and 0.72 with the most probable value 0.78. Burton ('35) made similar estimates on his subjects and arrived at the

conclusion that S lay, within wide limits, between 0.7 and 0.9. He also correctly pointed out that S would vary considerably in fat persons and in thin persons.

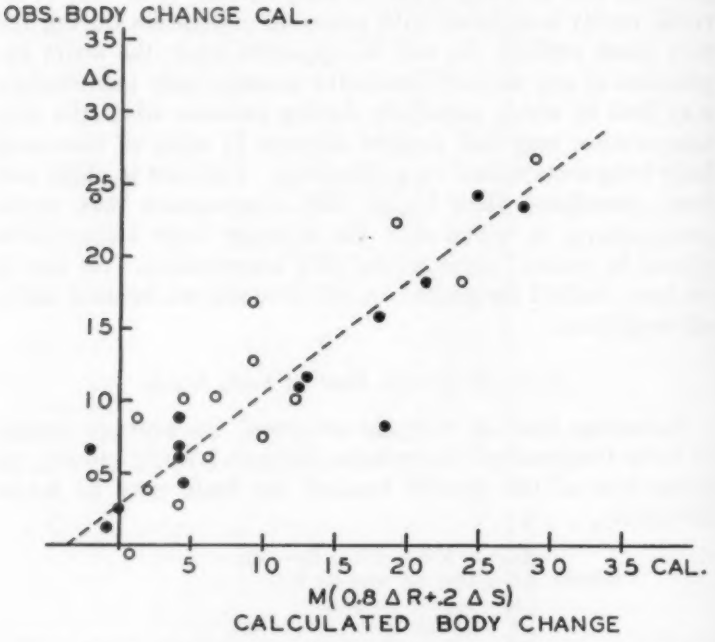

Fig. 5 Graphical estimation of specific heat of body from measured body heat change and measured body thermal change.

Heat production

Figure 3 summarizes the results on the subjects D and H. They bring out clearly the difference between heat production and heat elimination. The blank columns to the left show the heat production as determined from the oxygen consumption, nitrogen elimination, and R.Q., using the method of indirect calorimetry. During the periods of observation which have been called basal, the heat production of E.F.D.B. was 69 cals. per hour $\pm$ 3 cals., or 34.9 cals. per

square meter per hour. This, according to the Aub and Du Bois standards, is —7%. For J.D.H. the heat production was 62 cals. per hour ± 3 cals., or 35.0 cals. per square meter per hour. This is, according to the same standards, —12%. In the range of temperatures down to 22°C. it was possible for both subjects to remain in a basal state for about 2 hours, that is, during a preliminary and first experimental period. Actually, the heat production tended to decrease slightly in cold atmospheres, possibly in accord with van't Hoff's law. Temperatures up to 35°C. seemed to have no effect upon heat production.

In cold experiments the periods immediately preceeding a chill were not considered basal if the subjects were restless. During this time the subjects usually felt under considerable mental tension although the effort required to prevent the onset of chill was not found to increase oxygen consumption. At times, during some of these periods, involuntary muscular contractions occurred. In all cases in which increased heat production occurred, muscular 'tensing' or actual shivering appeared, and no increase in oxygen consumption as evidence of a purely chemical response to the exposure to cold (Rubner's chemical regulation of body temperature) was observed.

Heat elimination

Heat elimination, shown by the right hand columns in figure 3 with shaded areas, is equal to the heat production only in the relatively narrow neutral zone between 28°C. and 32°C. The thermal conditions of the atmosphere in this zone with about 30% relative humidity are such as to provide a natural escape for the body heat, under basal conditions, at exactly the rate of its production. In this 'thermally neutral zone' the subjects are comfortable throughout the experiment, although any amount of activity will usually produce sweating.

As the environmental temperature is lowered (down to 22°C.) the heat elimination becomes larger than the heat

produced by about 3 cals. per hour for each degree drop in temperature. The body temperature therefore falls as is evidenced by the rapid fall in skin temperature and the slow drop in rectal temperature. If 15 kg. of tissue falls 1°C. it will give off 8 to 12 calories, depending on the relative content of water (Sp.Ht. = 1.0) and fat (Sp.Ht. = 0.5) in the skin. The 8 to 12 calories thus eliminated were not produced by the metabolism during the basal periods.

The heat elimination also increases with rising environmental temperatures. This would be expected, since the body will absorb energy from the environment at temperatures greater than 35°C. However, no experiments were made at environmental temperatures over 35°C. as the calorimeter absorber mechanism is not adequate for the removal of such large amounts of moisture. The rise in heat loss between 31°C. and 35°C. is probably due to slight overcooling by the sweat glands.

The actual number of calories lost from the body through vaporization, V, convection, C, and radiation, R, are also shown in figure 3. Radiation, which depends solely upon the skin surface area and temperature and the environmental objects' temperature, increases from 0 at 35°C. up to more than the total heat production at 22°C. Vaporization decreases from about 80 calories per hour at 35°C. to from 15 to 19 calories per hour at 22°C.

Convection, which is most difficult to measure directly, is arrived at by the difference between the total heat absorbed by the calorimeter and the loss due to radiation. The convection loss is small, ranging from 8 to 16 cals., for nude subjects under basal conditions. The convection loss does not seem to depend to any great extent upon the temperature of the environment except at air temperatures near the skin temperature. Convection loss from inanimate objects is known to depend upon the size, shape, surface and position relative to other objects as well as upon difference in temperature between objects and air and air movement. In these experiments the natural air movement was so small that it

could scarcely be felt by a naked man. The position of the man in the box was such as would hamper free air movement, a fact which would help account for small convection losses. By far the most important factor in convection in our experiments is air movement. Convection is markedly increased by the slightest movement on the part of the man and even the movements of the ribs in breathing and the currents of air in and out of the nostrils cause some turbulence not found with inanimate objects. Slight movements of the extremities which scarcely affect the metabolism cause increased convection. Calorimeter humidity and the vaporizing of water from the skin have no demonstrable effects on convection. Rapid movements of the subject may cause convection to rise to almost as high a value as that due to the blast of air from an electric fan.

The percentages of heat lost through vaporization, convection, and radiation are shown in figure 6. It will be seen that the curves for vaporization and radiation are almost complementary except for the fact that vaporization never becomes zero. Radiation has its highest values at about 22°C. to 23°C. where it amounts to about 70% of the total heat loss. With increasing temperature radiation plays a smaller role and becomes zero at 35°C. Radiation is equal to vaporization in the upper 'neutral zone,' at 31°C.

Convection remains about constant, decreasing in importance in cold environments and in warm environments. The reason for the decrease in cold temperatures may be due to the fact that the subjects were concentrating on remaining quiet in effort to ward off the onset of chill. The rise in convection in the 'neutral zone' is partly due to increased ventilation in the calorimeter to take care of the moisture. The decrease in the proportion of heat loss due to convection in warmer environments is due to the smaller gradients between skin and air. Convection becomes zero at 35°C.

It should be noticed that radiation has its maximum proportion of heat loss and convection its minimum when the subject is lying motionless, i.e., in the basal state. Almost

any other condition tends to decrease the porportion due to radiation and increase that due to convection.

The limited ability of the vaso-constrictor mechanism of the body to provide sufficient protection against cold is demonstrated by the fact that the skin temperature falls only one degree when the environment has fallen two degrees. The

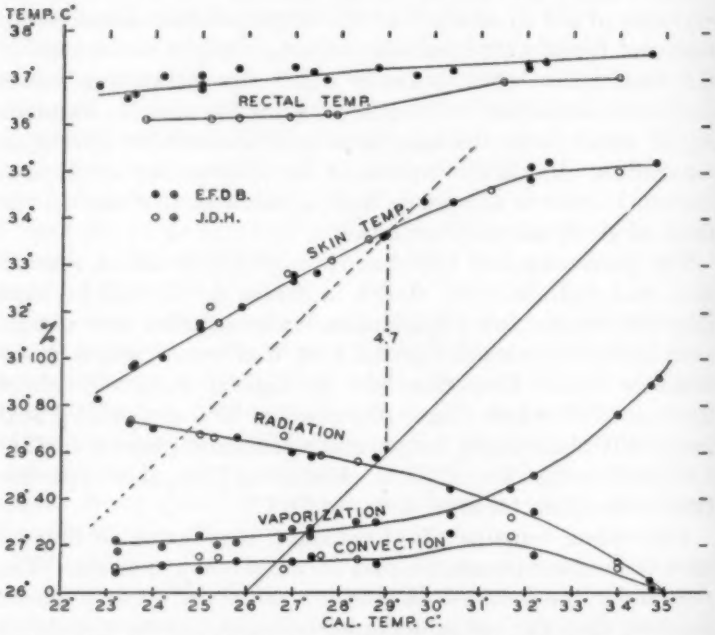

Fig. 6 Effect of calorimeter temperature upon rectal temperature, average skin temperature, and percentages of heat loss from radiation, vaporization and convection.

thermal gradient from the skin to the environment, at the lower temperature limit of the neutral zone, is 4.7°C. This is the maximum gradient over which the body can maintain its temperature without increase in heat production. Therefore, as the environmental temperature is lowered, the skin should drop so as to maintain a gradient no greater than this,

and should follow the dashed line in figure 4. The height of the true skin temperature above the dashed line is proportional to the rate at which the body temperature is falling.

Pulse, nitrogen elimination and total R.Q.

An increase in heart rate was observed in both subjects as the temperature of the experimental environment was raised. Subject D, who has a slow pulse, evidenced a linear rise from about 52 to 59 beats per minute. Subject H showed a rise from about 52 to 66. This is perhaps associated with the dilation of the skin vessels at the higher temperatures.

An observed fall in nitrogen elimination with increasing calorimeter temperature from about 0.7 to 0.3 gm. per hour is perhaps due in part to increased sweating, and yet it is apparent at temperatures where vaporization was changing but little. No attempt was made to control the protein intake the day before the experiment. Under similar dietary conditions in previous years control D, when studied in the calorimeter, eliminated rather uniformly about 0.5 gm. nitrogen per hour. Although Swift ('32) reported a similar effect, the evidence is not sufficient to indicate any specific change in nitrogen metabolism at varying temperatures. No temperature effect on the total R.Q. could be found.

SUMMARY AND CONCLUSIONS

It has been possible for the first time to measure quantitatively the total heat loss and the proportions due to radiation and convection from men exposed to various atmospheric conditions.

Two normal men were studied naked under basal conditions in the respiration calorimeter of the Russell Sage Institute of Pathology at temperatures between 22°C. and 35°C. The heat lost in vaporization was measured by weighing the water. The heat of radiation was determined by means of a Hardy radiometer. Convection was found by difference. Calculations were based on heat elimination and not heat production. Heat storage, which was not a factor in the

basic calculations, was estimated from rectal and surface temperature changes and was measured by the difference between direct and indirect calorimetry. Between 22°C. and 35°C. the average temperature of the skin lay about midway between that of the air and that of the internal parts of the body. At the lower temperatures the surface layer of the body cooled rapidly and heat elimination greatly exceeded heat production. Radiation accounted for about 70% of the total loss at 22°C. to 26°C., but this percentage fell rapidly to zero as skin and air temperatures approached each other. Vaporization dissipated 18% to 30% of the heat at the lower air temperatures but accounted for about 100% at 35°C. Convection remained fairly uniform at about 15% until the air temperature rose above 32°C. Convection is significantly increased by slight movements of the body or the air.

The basal metabolism of both naked subjects was level throughout the range of air temperatures from 22°C. to 35°C. At the lower temperatures the temperature of the skin and subcutaneous tissues dropped rapidly. After about 2 hours the men felt distinctly chilly and there was a tensing of the muscles which raised the metabolism slightly. After 10 or 15 minutes this was followed by a shaking chill. The fact that metabolism was not increased until a few minutes before the chill throws doubt on the existence of Rubner's 'chemical regulation.' At the higher temperatures increased sweating took care of the heat loss when radiation and convection were abolished.

LITERATURE CITED

ALDRICH, L. B. 1928 A study of body radiation. Smithsonian Misc. Coll., vol. 81, no. 6.

BARR, D. P., AND E. F. DU BOIS 1918 Clinical Calorimetry 28, The metabolism in malarial fever. Arch. Int. Med., vol. 21, p. 627.

BARKER, A. H. 1932 The principles of calculation of low temperature radiant heating. Domestic Eng., vol. 52, p. 3.

BENEDICT, C. G., F. G. BENEDICT AND E. F. DU BOIS 1925 Some physiological effects of hot air baths. Am. J. Physiol., vol. 73, p. 429.

BOHNENKAMP, H., AND H. W. ERNST 1931 Untersuchungen zu den Grundlage des Energie, und Stoffwechsels. II Über die Strahlungsverlüste des Menschen. Arch. f. d. ges. Physiol., Bd. 228, S. 63.

BURTON, A. C. 1934 A new technique for the measurement of average skin temperature over surfaces of the body and changes in skin temperature during exercise. J. Nutrition, vol. 7, p. 481.

———— 1935 Human Calorimetry II. The average temperature of the tissues of the body. J. Nutrition, vol. 9, p. 261.

BURTTON, A. C., AND H. C. BAZETT 1936 A study of the average temperature of the tissues, of exchanges of heat and vasomotor responses in man by means of a bath calorimeter. Am. J. Physiol., vol. 117, p. 36.

DEIGHTON, T. 1933 Physical factors in body temperature maintenance and heat elimination. Physiol. Rev., vol. 13, p. 427.

DU BOIS, E. F. 1936 Basal Metabolism in Health and Disease, 3rd edition, Lea and Febiger, Philadelphia.

HARDY, J. D., AND E. F. DU BOIS 1938 The technique of measuring radiation and convection. J. Nutrition, vol. 15, p. 461.

HOUGHTEN, F. C., W. W. TEAGUE, W. E. MILLER AND W. P. YANT 1935 Heat and moisture losses from the human body and their application to air conditioning problems. Am. Soc. Heat and Vent. Eng. Trans., vol. 35, p. 245.

LUSK, G. 1928 The Science of Nutrition, 4th ed., W. B. Saunders Co., Philadelphia.

SWIFT, R. W. 1932 The effects of low environmental temperature upon metabolism. II. J. Nutrition, vol. 5, p. 277.

WILEY, F. H., AND L. H. NEWBURGH 1931 The relationship between the environment and the basal insensible loss of weight. J. Clin. Invest., vol. 10, p. 689.

WINSLOW, C.-E. A., L. P. HERRINGTON AND A. P. GAGGE 1936 The determination of radiation and convection exchanges by partitional calorimetry. Am. J. Physiol., vol. 16, p. 669.

———— 1937 a Physiological reactions of the human body to varying environmental temperatures. Am. J. Physiol., vol. 120, p. 1.

———— 1937 b Physiological reactions of the human body to various atmospheric humidities. Am. J. Physiol., vol. 120, p. 288.

YAGLOU, C. P. 1926 The thermal index of atmospheric conditions and its applications to sedentary and to industrial life. J. Indust. Hyg., vol. 8, p. 5.

THE EFFECT OF UREA ON THE HUMAN RESPIRATORY EXCHANGE AND ALVEOLAR CARBON DIOXIDE [1]

THORNE M. CARPENTER

Nutrition Laboratory, Carnegie Institution of Washington, Boston

(Received for publication November 26, 1937)

Urea constitutes the major nitrogenous excretion in the urine in the metabolism of protein and, therefore, has been the subject of many studies as to whether the transference of this substance from the blood into the urine is the cause of the specific dynamic action of protein. Lublin ('28) and Borsook and Winegarden ('31 a, b, c) came to the conclusion that there was an increased energy output due to the ingestion of urea, whereas Tangl ('11), Lusk ('12), Grafe ('15), Kocher and Torbert ('32), di Frisco ('33), Eaton, Cordill and Gouaux ('35), and Rajzman ('36) found negative results so far as any effect of ingestion of urea on the total metabolism is concerned. Ingestion of urea by mouth should cause no change in the respiratory quotient, as no oxidation is involved in its passage in an unchanged form through the body. Adolph ('25), however, found that there was an increase in the alveolar carbon-dioxide tension when urea was given to human subjects. In a general study of the various factors affecting the respiratory quotient, this investigation has been made to determine whether with a change in the alveolar carbon dioxide there would be a change in the respiratory quotient and at the same time to add another study on the question of the effect of urea on the total metabolism.

[1] A preliminary report of this material was presented at the meeting of the American Chemical Society, Rochester, N. Y., on September 7, 1937.

Methods. The respiratory quotient was measured by the open circuit apparatus of Carpenter and Fox ('31) in one group of experiments and by the Benedict helmet ('30, '33) and open circuit apparatus in another group. When the apparatus of Carpenter and Fox was used, alveolar air samples were collected every 7 to 8 minutes in parallel with the respiratory exchange measurements by the arrangement of Carpenter and Lee ('33).

Procedure. The subject was H. C. W., 22 years of age, 68.0 kg. in weight, and 175 cm. in height. The experiments were all made with him in a sitting position and in the post-absorptive condition. After the preliminary rest, measurements were made in three periods of 15 minutes' duration each, and the dose for the day was then given to the subject. When the apparatus with mouthpiece was used, the ingestion of the dose was followed by three 15-minute periods of measurement out of each succeeding hour (the first three quarter-hours) until a total of 3½ hours had elapsed after the dose was given. In one series of urea experiments the sequence of periods was changed in that only two 15-minute periods of measurement immediately followed the dose and then groups of three 15-minute periods followed which were separated by 15-minute intervals without measurement. In this way the periods omitted in the grouping first described were covered in this particular series. Two series were also made, one with water and one with urea and water, in which only one 15-minute period of measurement was made in each half hour and the subject drank water and urinated every half hour. When the helmet was used, there were three base-line periods as in the mouthpiece experiments, but after the dose the measurements of the respiratory exchange were made continuously in 15-minute periods without interruption for 3 hours. By this procedure the effect of interruption of observations was avoided and the true continuous changes in the gaseous exchange and the respiratory quotient were determined. Four types of experiments were made. The first was with no dose, in which the whole routine was carried out

as in the other groups; the second was with 400 or 500 cc. of water to serve as a control for the urea experiments; and the third and fourth series were with 30 and 40 gm. of urea in 400 and 500 cc. of water, respectively.

ALVEOLAR CARBON DIOXIDE

The results of the determinations of the alveolar carbon dioxide are given in table 1. The table includes the number

TABLE 1

Human alveolar carbon dioxide in experiments with no dose, water and urea

	NO DOSE	400 CC. WATER	30 GM. UREA [1]	40 GM. UREA [2]	40 GM. UREA [2]
	%	%	%	%	%
Number of experiments	4	4	5	3	3
Base line	5.73	5.94	5.92	5.69	5.72
Minutes after ingestion					
0– 15	5.72	5.78	5.96	5.91	5.88
15– 30	5.70	5.75	6.12	6.07	6.05
30– 45	5.65	5.83	6.21	6.07	..
45– 60	..	..	..	..	6.01
60– 75	5.75	5.93	6.09	5.97	5.97
75– 90	5.77	5.88	6.06	6.06	5.98
90–105	5.73	5.93	6.07	6.03	..
105–120	..	..	..	..	5.92
120–135	5.75	5.75	5.91	5.83	5.82
135–150	5.68	5.77	5.89	5.86	5.81
150–165	5.66	5.81	5.83	5.75	..
165–180	..	..	..	..	5.82
180–195	5.68	5.78	5.86	5.81	5.86
195–210	5.71	5.77	5.87	5.75	5.78

[1] In 400 cc. of water.
[2] In 500 cc. of water.

of experiments in each series, the average base-line values for the three 15-minute periods preceding the dose for the day, and the averages by 15-minute periods for 3½ hours following the dose. In the first four series, the periods of 45 to 60, 105 to 120, and 165 to 180 minutes had no observations of the alveolar air as the subject rested in these periods. In the last column the series covering these missing periods is given.

With no dose there was a uniform alveolar carbon dioxide throughout the series, as the range was only from 5.65 to 5.77%, including the base-line value. The original protocols show that even in the average values at every 7 to 8 minutes the range was only 5.61 to 5.82%.

In the series with 400 cc. of water there was a significant fall in the alveolar carbon dioxide in the first three quarter-hours after the water was taken. In the second hour there was a return to practically the base-line value. In the third hour and the first half of the fourth hour there was again a fall. These changes are significant, as the range of the seven average values for every 7- to 8-minute interval in the base-line three quarter-hours was from 5.85 to 6.02%, whereas in the seven average values of the first hour after the dose the range was from 5.71 to 5.88%. Also, in the last 1½ hours, the range of twelve average values at 7- to 8-minute intervals was from 5.71 to 5.89%. At the present time no satisfactory explanation can be given as to the cause of this change in the alveolar carbon dioxide after the ingestion of 400 cc. of water.

With 30 gm. of urea there was a definite rise in the alveolar carbon dioxide that began in the second quarter-hour after ingestion and lasted through the second hour. The highest average value was 6.21% in the third quarter-hour as compared with 5.92%, the average of the three quarter-hours of the base-line group. At the twenty-third, thirty-second, thirty-eighth and forty-seventh minutes of the measurements after ingestion the average values were 6.16, 6.20, 6.14 and 6.28%, so that the rise shown by the average values 6.12 and 6.21 in the second and third quarter-hours is significant. With 40 gm. of urea there was an even more marked rise, for, although numerically the average values after ingestion were close to those after 30 gm., the base-line value with 40 gm. was 5.69 compared with 5.92% with 30 gm. The rise continued for 2½ hours. When the spacing of the periods was changed so that the omitted quarter-hours in the first series with 40 gm. were covered, the percentage of alveolar carbon dioxide rose to the same level and the rise lasted about the

same length of time. It is evident from these two series with 40 gm. of urea that the 15-minute intermission produced no effect on the alveolar carbon dioxide in the periods immediately following the intermission. The rise in alveolar carbon dioxide after the ingestion of urea by man found by Adolph ('25) is, therefore, fully confirmed.

TABLE 2

Average changes from the base line in the human R.Q. and oxygen absorption in no-dose, water and urea experiments. (Open circuit apparatus with mouthpiece)

	NO DOSE		400 CC. WATER		30 GM. UREA [1]		40 GM. UREA [2]	
	R.Q.	O_2 per minute	R.Q.	O_2 per minute	R.Q.	O_2 per minute	R.Q.	O_2 per minute
Number of experiments	4	cc. 4	4	cc. 4	5	cc. 5	3	cc. 3
Base line	0.81	263	0.80	263	0.82	254	0.80	254
Minutes after ingestion								
0– 15	0	—3	0	+8	—0.02	+9	—0.02	—4
15– 30	+0.01	—2	+0.01	—3	—0.02	0	—0.01	—6
30– 45	+0.01	—8	+0.01	+1	0	+2	0	—6
60– 75	+0.01	—3	0	—1	+0.01	+6	+0.01	—3
75– 90	+0.01	—1	+0.01	—2	+0.02	—1	+0.04	—3
90–105	+0.01	—1	+0.02	—1	+0.02	—5	+0.04	+1
120–135	+0.01	—5	0	0	0	+3	+0.02	—11
135–150	0	—7	0	—1	+0.01	—2	+0.03	—8
150–165	+0.01	—2	+0.01	—5	+0.02	0	+0.03	—4
180–195	—0.02	—7	—0.01	—6	—0.01	+8	0	—13
195–210	—0.01	—8	+0.01	—8	—0.01	+9	+0.02	+4

[1] In 400 cc. of water.
[2] In 500 cc. of water.

OXYGEN ABSORPTION

The effect of ingestion of urea on the total metabolism and, therefore, on the oxygen absorption is a subject that has occupied the attention of a number of workers, and the results obtained by them have not agreed with one another. Although the primary purpose of the study here reported was not to determine the effect of urea on the oxygen absorption but rather the relation of the changes in the respiratory quotient to those of the alveolar carbon dioxide, the results are uniformly so definite that it has seemed advisable to include

them. The values obtained with the mouthpiece are given in table 2, and those with the Benedict helmet in table 3. Briefly stated, the results showed no rise in the oxygen absorption after the ingestion of 30 or 40 gm. of urea for a period of 3 to 3½ hours. There was a slight fall after urea in the second and third hours, although the maximum change from the base-line value was only 5%, that is, —13 cc. in the period of 180 to

TABLE 3

Average changes from the base line in the human R.Q. and oxygen absorption in no-dose, water and urea experiments. (Benedict helmet apparatus)

	NO DOSE		400–500 CC. WATER		30 GM. UREA [1]		40 GM. UREA [2]	
	R.Q.	O_2 per minute	R.Q.	O_2 per minute	R.Q.	O_2 per minute	R.Q.	O_2 per minute
Number of experiments	3	cc. 3	3	cc. 3	2	cc. 2	3	cc. 3
Base line	0.81	248	0.83	245	0.83	243	0.80	248
Minutes after ingestion								
0– 15	—0.01	—2	0	—2	—0.01	—1	—0.02	+1
15– 30	+0.01	+2	+0.02	—7	0	+3	+0.01	—3
30– 45	+0.01	—4	+0.02	—8	+0.01	+5	+0.01	—2
45– 60	+0.01	—3	+0.01	+1	+0.04	+5	+0.02	—1
60– 75	+0.01	—4	+0.01	—3	+0.02	—6	+0.03	+1
75– 90	+0.01	+1	+0.01	—4	+0.03	—3	+0.04	—5
90–105	+0.02	—4	0	+1	+0.02	—9	+0.03	—3
105–120	+0.01	0	+0.01	—6	+0.03	—8	+0.03	—1
120–135	+0.02	—10	0	—2	+0.03	—2	+0.03	—7
135–150	+0.02	—4	0	+1	+0.04	—9	+0.03	—5
150–165	+0.01	0	+0.01	0	+0.03	+1	+0.03	—8
165–180	+0.01	—5	+0.01	0	+0.04	—5	+0.01	+1

[1] In 400 cc. of water.

[2] In 500 cc. of water.

195 minutes after ingestion of 40 gm. of urea in the series with the mouthpiece. These experiments differ from preceding studies of this nature with man in that the measurements were not made in single isolated periods but rather in an uninterrupted series of three periods with the mouthpiece and twelve periods with the helmet. The effect of preceding minor activity on the oxygen absorption is thus avoided, particularly in the series of twelve periods with the helmet apparatus.

RESPIRATORY QUOTIENT

The principal reason for this investigation was to ascertain the course of the respiratory quotient when there was a change in the alveolar carbon dioxide that was not produced by artificial means, such as inhaling high percentages of carbon dioxide or by forced overventilation, and that was unaccompanied by any metabolic oxidation process. The interpretation of the changes in the respiratory quotient for a period of 3 to 4 hours after the ingestion of a substance is dependent on the changes that would occur in the same period of time with no dose or with a control with the same volume of water as was used for the ingestion of the substance to be studied. In the series with no dose with the mouthpiece, the changes from the base-line value in 3½ hours varied from + 0.01 to — 0.02. Although the majority are plus in sign, the average is so small and the variations in the individual forty-four periods of the experiments are so large (— 0.05 to + 0.04) that these slight increases cannot be considered significant. In the twelve periods with the helmet following the base-line group, the changes are all positive but one, but the largest is only + 0.02. The slight increases were caused by the almost uniform rise in the quotient in one experiment as compared with the changes in both directions in the other two experiments. With 400 and 500 cc. of water there was little change in either direction in the majority of the periods with the mouthpiece, but with the helmet in eight out of twelve periods there was a slight rise in respiratory quotient. In this series the positive changes are brought about mathematically by the almost uniform rises in one of the three experiments.

With 30 gm. of urea there was at first a slight fall in the respiratory quotient in the series with the mouthpiece. This may or may not be significant in comparison with the changes in alveolar air. One would expect that with a shift in the alveolar air toward an increasing amount of carbon dioxide there would be temporarily a lowering of the respiratory quotient. In the last two periods of the second hour there was

a slight rise of 0.02. Similarly in the third hour there was a rise of 0.02 in the third period. In the last two periods of the experiment the respiratory quotient had fallen to below the original base line. In the series with the helmet after the first three quarter-hours there was a definite rise in the respiratory quotient throughout the whole series, that is, over a period of 2¼ hours. This rise is so large, reaching in several instances 0.04, that there can be little question that there was a significant increase in the respiratory quotient. The results with the helmet were more definite because there was no interruption and therefore no omission of any variation which might take place due to the shifting from a condition of breathing through a mouthpiece to one of rest. In the series with 40 gm. with the mouthpiece, the results were somewhat similar for the first hour to those with 30 gm. of urea. However, in the second and third hours there was a definite increase in the respiratory quotient in all but one period and one cannot conclude that the increase has been entirely eliminated in the last half-hour of the experiments. With the helmet the increase on the whole was not quite so large after 40 gm. as after 30 gm. but was definite in the periods beginning 45 minutes after ingestion and ending 165 minutes after ingestion. There was thus a definite rise in the respiratory quotient after the ingestion of 30 gm. or 40 gm. of urea.

URINARY NITROGEN

In all the experiments, the urine was collected from the time the subject arrived at the laboratory until after the experiment was completed, unless the subject desired to void urine before the end of the experimental period.

For an average period of a little over 5 hours the average amount of nitrogen in the urines in seven no-dose experiments was 1.98 gm. and in eight experiments with water it was 2.26 gm. The average urinary nitrogen in experiments with 30 gm. of urea was 6.49 gm. and with 40 gm., 7.56 gm. The periods of time represented by the urea experiments were also about 5 hours. From the differences between the experiments

with and without urea it can be estimated that about 30% of the ingested urea was eliminated in the 3 to 4 hours after ingestion. In two special series of experiments, one with 500 cc. of water and the other with 40 gm. of urea in 500 cc. of water, the urine was collected every ½ hour for 4 hours after the ingestion of the dose. The subject in these series was also given 200 cc. of water at each half-hour interval. In these urine samples, total nitrogen, urea, and ammonia were determined by the methods of Folin ('34) and associates. The ammonia-nitrogen in the control series in the 4 hours was 153, 93, and 141 mg. and each experiment did not show a wide variation in the eight half-hour periods. The amounts averaged 6.8, 3.0, and 5.3% of the total nitrogen, respectively. In the three experiments with 40 gm. of urea the total ammonia-nitrogen was 112, 97, and 98 mg. and averaged 1.4, 1.2, and 1.1% of the total nitrogen. There was thus a slightly lower ammonia elimination after the ingestion of urea. The urea-nitrogen in the water series was 1.84, 2.54, and 1.98 gm. and constituted 82, 83, and 75% of the total nitrogen. In the three experiments with 40 gm. of urea, the urea-nitrogen was 7.46, 7.48, and 7.54 gm. and formed 92, 91, and 88% of the total nitrogen. The maximum elimination of urea-nitrogen was in the second ½ hour after ingestion and was 1.48, 1.39, and 1.36 gm. This was the period of maximum change in the alveolar carbon dioxide. The average of the urea-nitrogen in the three water experiments was 2.12 gm. and in the three urea experiments, 7.49 gm. The difference between the two series was 5.37 gm., which corresponds to about 12 gm. of urea. The total nitrogen eliminated in the three water experiments was 2.25, 3.07 and 2.64 gm. In the urea experiments the total nitrogen elimination was 8.10, 8.20, and 8.60 gm. The averages of the two series are 2.65 and 8.30 gm., respectively. The difference between the averages is 5.65, corresponding to 12 gm. of urea. Of the 40 gm. of urea ingested there was about 30% eliminated in the urine in approximately 4 hours.

DISCUSSION

In the digestion of protein and subsequent metabolism, urea is formed in quantities varying according to the rate at which the various processes take place. One would expect, therefore, that after the ingestion of meat there would be sometime during its metabolism a considerable increase in urea in the blood. Therefore, one would look for a change in either the alveolar carbon dioxide or the carbon-dioxide content of the blood during the metabolism after ingestion of protein. Hasselbalch ('12) found a lowering of the alveolar carbon dioxide after administering a diet of meat and fat for several days in contrast to the increase noted with an alkaline vegetarian diet. Bischoff and co-workers ('34) found on two occasions that after the ingestion of a pound of steak there was a fall of 3 m. Mol. in the plasma bicarbonate at the eighth hour after ingestion. Wright ('36) states that a meat diet gives rise to an excess of acid radicals, especially H_3PO_4 and H_2SO_4 derived from the oxidation of phosphorus and sulphur of the protein, and the alveolar carbon dioxide is found to fall. However, in all these cases it is probable that these effects are not the immediate results of ingestion of protein but rather ones which come on in the final stages of the formation of the end products of oxidation. Chanutin ('21) found that after 940, 810, and 1000 gm. of meat ingested by a dog there was a marked rise in the carbon-dioxide-combining power of the blood even in the first hour and states that it is possible that the interpretation of Erdt ('15) was correct in that the rise in the carbon-dioxide tension following the meal is due to the secretion of hydrochloric acid by the gastric juices. Aub and Du Bois ('17) found with two of four subjects in periods beginning $1\frac{1}{2}$ to 2 hours after ingestion of approximately 600 gm. of beef, respiratory quotients as high as 0.87 to 0.91. The other two subjects and all subjects in the basal condition gave respiratory quotients ranging from 0.77 to 0.86. McClellan, Spencer, Falk and Du Bois ('28), however, found respiratory quotients that averaged as a whole 0.75 when the subjects were given meat meals. Their basal quotients ranged

between 0.72 and 0.78, and the average was 0.76. Thus there was no effect of the ingestion of meat upon the respiratory quotient. These subjects, however, had been existing on either a low carbohydrate diet such that mild acidosis was produced or they were near the borderline for the condition to produce acidosis. Porges, Leimdörfer, and Markovici ('11) found a rise in the carbon-dioxide tension in the blood after meals and ascribed it to the gastric secretion. Since that time there have been many studies on the changes in alveolar carbon dioxide, carbon-dioxide content of the blood, and the alkaline tide in relation to the ingestion of food and the gastric secretion. The general opinion is that there is a relationship between the gastric secretion involving the formation of hydrochloric acid and the other factors mentioned. Apperly ('36), in fact, has recently come to the general conclusion that the alkaline reserve of the blood and the gastric secretion among different individuals are related. It would, therefore, appear that not only may there be a change in the alveolar air as a result of the presence of urea in the blood after the ingestion of protein but also a change in the alveolar carbon dioxide in the early part of the period of time after ingestion of protein due to gastric secretion. In the experiments here reported, when there was a change of alveolar carbon dioxide which was brought about in metabolic processes there was also an effect upon the respiratory quotient so that for a period of time the quotient was not actually as high as it should have been, and subsequently it rose, due to the release of the carbon dioxide in the blood and the return of the alveolar carbon dioxide to the pre-ingestion level. The changes in the alveolar carbon dioxide and the subsequent changes in the respiratory quotient must have been due to the presence of urea in the blood itself. Therefore, there would be no reason to infer that such a change did not take place in the normal formation of urea and its release into the blood during the process of metabolism of protein. It must be recognized that the respiratory quotient after the ingestion of protein for a long period of time does come to the normal empirical quotient

which has been used for many years, but in any interpretation of the periodic changes that take place after the ingestion of protein or various fragments of protein, it must be recognized that the changes that may occur are influenced somewhat by the temporary disturbance of the carbon-dioxide content of the blood, with the subsequent release of carbon dioxide to the outcoming air.

SUMMARY

The respiratory exchange of a human subject was determined for 3 or 3½ hours after ingestion of 400 and 500 cc. of water or 30 or 40 gm. of urea in 400 and 500 cc. of water, respectively. Two methods were used for the determination of the respiratory exchange, 1) an open circuit apparatus with mouthpiece, and 2) an open circuit apparatus with helmet. When the apparatus with the mouthpiece was used, simultaneous determinations of the alveolar carbon dioxide were made every 7 to 8 minutes. There was no effect upon the total metabolism as a result of the ingestion of 30 or 40 gm. of urea when compared with the measurements for the same periods of time in no-dose experiments and those with water. In the experiments with water there was a slight drop in the alveolar carbon dioxide for most of the 3½ hours after the ingestion. With 30 and 40 gm. of urea there was a marked rise in the alveolar carbon dioxide for 2 to 2½ hours. The respiratory quotient was not changed significantly in either the no-dose experiments or the experiments with 400 and 500 cc. of water. With 30 and 40 gm. of urea there was a significant rise in the respiratory quotient beginning at the fourth or sixth quarter-hour after ingestion and lasting until the end of the third hour after ingestion. The study shows that along with an alteration in the alveolar carbon dioxide there is an alteration in the respiratory quotient as the result of the alkalosis following the ingestion of urea. As the alveolar carbon dioxide also rises due to the gastric secretion after the ingestion of protein, it is pointed out that there may be two causes for alterations in the respiratory quotient from the

true respiratory quotient after the ingestion of protein, one the gastric secretion containing hydrochloric acid and the other the urea that ultimately results as a metabolic product of the transformations of protein in the body.

The determinations of the respiratory exchange and alveolar air were made by Mr. B. James, assisted by Mr. R. E. Murray, who also made the analyses of the urines.

LITERATURE CITED

ADOLPH, E. F. 1925 Alkalosis produced by ingesting urea. Am. J. Physiol., vol. 72, p. 185.

APPERLY, F. L. 1936 Gastric acidity and its significance. Lancet, vol. 106, p. 1234.

AUB, J. C., AND E. F. DU BOIS 1917 Clinical calorimetry. XXI. The basal metabolism of dwarfs and legless men with observations on the specific dynamic action of protein. Arch. Intern. Med., vol. 19, p. 840.

BENEDICT, F. G. 1930 A helmet for use in clinical studies of gaseous metabolism. New England J. Med., vol. 203, p. 150.

———— 1933 Der Helm-Respirationsapparat in seinen verschiedenen Formen. Abderhalden's Handbuch d. biolog. Arbeitsmethoden, Abt. IV, Teil 13, S. 465.

BISCHOFF, F., W. D. SANSUM, M. L. LONG AND M. M. DEWAR 1934 The effect of acid ash and alkaline ash foodstuffs on the acid-base equilibrium of man. J. Nutrition, vol. 7, p. 51.

BORSOOK, H., AND H. M. WINEGARDEN 1931 a The work of the kidney in the production of urine. Proc. Nat. Acad. Sci., vol. 17, p. 3.

———— 1931 b The energy cost of the excretion of urine. Proc. Nat. Acad. Sci., vol. 17, p. 13.

———— 1931 c On the specific dynamic action of protein. Proc. Nat. Acad. Sci., vol. 17, p. 75.

CARPENTER, T. M., AND E. L. FOX 1931 An apparatus for continuous short period measurements of human respiratory exchange during rest and muscular work. Arbeitsphysiol., Bd. 4, S. 527.

CARPENTER, T. M., AND R. C. LEE 1933 The parallel determination of the respiratory quotient and alveolar air of man in the post-absorptive condition. J. Nutrition, vol. 6, p. 37.

CHANUTIN, A. 1921 Animal calorimetry. XX. The influence of the ingestion of meat and of glycine and alanine upon the carbon dioxide-combining power of blood plasma. J. Biol. Chem., vol. 49, p. 485.

EATON, A. G., S. C. CORDILL AND J. L. GOUAUX 1935 Is the work of the kidney, due to the excretion of urea, a factor in specific dynamic action? Am. J. Physiol., vol. 113, p. 37; J. Nutrition, vol. 10, p. 429.

ERDT, H. 1915 Alveolargasanalysen. III. Mitt. Die Tagesschwankungen der Kohlensäurespannung der Alveolarluft und ihre Ursachen. Deutsch. Arch. f. klin. Med., Bd. 117, S. 497.

FOLIN, O. 1934 Laboratory Manual of Biological Chemistry, 5th ed., New York.

DI FRISCO, S. 1933 Sull-azione dinamico specifica degli alimenti. III. Lavoro renale e metabolismo gassoso del complessivo organismo. Arch. Int. Pharm. et Therap., vol. 45, p. 255.

GRAFE, E. 1915 Beiträge zur Kenntnis der Ursachen der spezifisch-dynamischen Wirkung der Eiweisskörper. Deutsch. Arch. f. klin. Med., Bd. 118, S. 1.

HASSELBALCH, K. A. 1912 Neutralitätsregulation und Reizbarkeit des Atemzentrums in ihren Wirkungen auf die Kohlensäurespannung des Blutes. Biochem. Zeit., Bd. 46, S. 403.

KOCHER, R. A., AND H. C. TORBERT 1932 The effect of urea ingestion on the nitrogen partition of the urine at endogenous nitrogen level. J. Biol. Chem., vol. 95, p. 427.

LUBLIN, A. 1928 Über den Einfluss der Nierenarbeit auf den Gaswechsel des Menschen. Zeit. f. klin. Med., Bd. 109, S. 371.

LUSK, G. 1912 Animal calorimetry. III. Metabolism after the ingestion of dextrose and fat, including the behaviour of water, urea and sodium chloride solutions. J. Biol. Chem., vol. 13, p. 27.

MCCLELLAN, W. S., H. J. SPENCER, E. A. FALK AND E. F. DU BOIS 1928 Clinical calorimetry. XLIII. A comparison of the thresholds of ketosis in diabetes, epilepsy, and obesity. J. Biol. Chem., vol. 80, p. 639.

PORGES, O., A. LEIMDÖRFER AND E. MARKOVICI 1911 Über die Kohlensäurespannung des Blutes in pathologischen Zustanden. Zeit. f. klin. Med., Bd. 73, S. 389.

RAJZMAN, A. 1936 Action dynamique specifique et activité rénale. Arch. internat. de physiol., T. 43, p. 423.

TANGL, F. 1911 Die Arbeit der Nieren und die "spezifisch-dynamische Wirkung" der Nährstoffe. Biochem. Zeit., Bd. 34, S. 1.

WRIGHT, S. 1936 Applied Physiology, 6th ed., Oxford University Press, New York, p. 447.

THE BASAL METABOLISM IN PREGNANCY

JOSEPH A. JOHNSTON
Department of Pediatrics, Henry Ford Hospital, Detroit
AND
HELEN A. HUNSCHER, FRANCES COPE HUMMEL, MARY F. BATES, PRISCILLA BONNER AND ICIE G. MACY
Research Laboratory, Children's Fund of Michigan, Detroit

THREE FIGURES

(Received for publication November 9, 1937)

The course of the basal metabolism throughout pregnancy is well established, the largest group of observations being those of Rowe and Boyd ('32) who made serial determinations on seventy-seven women. They describe a fall in the energy requirement from a normal to a subnormal level during the third to the fourth month of gestation, the low level being reached in about 4 weeks. "From this point on, during the last 6 lunar months there is a steady increase in the basal metabolic rate amounting to 13% or more in excess of that conditioned by the gross increase in body weight."

The point has been at issue as to whether or not this rise could be explained as the heat production resulting from a new protoplasmic mass, or whether some glandular influence would not have to be invoked to explain the change. Sandiford and Wheeler ('24), analyzing the data from their single case and those in the literature, concluded that "the energy production of a unit mass of the mother's protoplasmic tissue remains unchanged throughout the course of pregnancy, and that such increases in the total heat production as occur are due to the increasing mass of active protoplasmic tissue consisting in large part of the fetal tissues and in lesser part of

maternal structures." Since Carpenter and Murlin ('11) had shown that just after birth the energy of the mother and child had shown no deflection, Sandiford and Wheeler argue that the fetus as well as the baby after birth should be regarded as a being independent of the mother and its heat production calculated on its own surface area which is proportional to its own active protoplasmic mass before and after delivery. Harding ('25) reviewed the entire subject and felt that the experiments recorded up to that time offered "no support to the idea that a special hyperactivity of the maternal thyroid occurs during pregnancy." He did note, however, that the calculations of Zuntz and of Carpenter and Murlin showed an increased metabolic rate in the mother, as distinct from the fetus, of about 4%. The method of approach taken to reach this conclusion—the calculation of the maternal and fetal areas independently and the combining of them into a so-called total effective area—is questioned by Rowe, who points out several flaws in the reasoning. First, it ignores wholly the lowering in heat production which takes place early in pregnancy. Secondly, when a large series is considered, and this total effective area is divided into the caloric output, while a fairly constant quotient is obtained, on close examination this value proves to show a definite progression defining a curvilinear relationship with a maximum approximately midway in the series. Thirdly, when curves are constructed in which there are plotted total calories, maternal calories, and the fetal areas, the data all show a linear change in basal rates but a curvilinear relationship of the fetal area, and hence a discrepancy in the attempt to compromise the two sets of data.

EXPERIMENTAL PROCEDURE

The energy data here recorded were obtained on a subject whose mineral metabolism was previously reported (Hummel et al., '37). The fasting metabolism was determined under basal conditions by the gasometer method. Gas analyses were made in duplicate or triplicate with the Haldane-Henderson apparatus, samples of approximately 9.5 cc. being used. Nitrogen balances were calculated from the analyses of food, urine,

feces, and breast milk. Calculations of the 'basal' metabolic mixture were made from the table of Lusk ('24). These are, of course, not accurate since the 24-hour urine was not subdivided and the figure used for the basal hour's nitrogen was one-twenty-fourth of the total. The error is, however, constant and we feel that the calculation brings out a point pertinent to this discussion.

FINDINGS

The energy data are recorded in table 1. Plotted graphically (fig. 1) the curve follows that of Rowe. When this curve is plotted against the cumulative nitrogen and sulfur balance a striking parallelism is suggested, which, however, is not maintained during the puerperium.

That the rise in metabolism is not incidental to an increased intake of food is evident from figure 2. While an attempt was made to have the food intake governed to a large extent by appetite and while there is a steady rise in food intake throughout the first four periods, from that point up to the delivery, the caloric intake is constant as is the nitrogen.

It is worth noting (fig. 3) that the rise in metabolism is not paralleled by an increase in urinary nitrogen, and that the metabolic mixture using one-twenty-fourth of the daily nitrogen as the basal hour shows a decline in the percentage of calories derived from protein.

COMMENT

Any attempt to interpret these findings as the relatively simple mathematical result of increased mass must ignore a number of well-established glandular changes concomitant with pregnancy and must make the assumption that these have occurred without influencing the maternal oxygen consumption. An increase in volume to the extent of two and one-half times the normal has been demonstrated in the case of the pituitary gland (Kolde, '12). The changes are almost limited to the anterior lobe. These changes are demonstrable between the third and fourth months.

TABLE 1

PERIOD	DATE	TOTAL CALS. PER HR.	SURFACE AREA	CALS./ SQ.M./HR.	BMR	RQ	EST.[1] TOTAL CALS.	BASAL CALS./ 24 HR.	METABOLIC MIXTURE					
									P		F		C	
									gm.	% cals.	gm.	% cals.	gm.	% cals.
1	3/16	58.7	1.51	38.9	− 3	0.79	57.0	1367	3.9	28.7	3.0	48.0	3.3	23.6
2	3/25	59.3	1.52	39.0	− 2	0.78	58.1	1396	3.9	26.0	2.9	46.0	3.3	28.0
4	4/ 1	60.0	1.52	40.1	± 0	0.84	60.0	1441	3.0	29.0	2.5	31.0	5.9	40.0
5	4/ 8	58.7	1.52	38.6	− 3	0.82	57.6	1408	3.2	22.4	2.9	46.5	4.4	31.0
7	4/15	63.7	1.53	41.6	+ 4	0.81	62.5	1528	3.1	20.4	3.7	53.0	4.1	26.6
8	4/22	60.9	1.53	39.8	± 0	0.81	59.6	1461	3.4	23.2	3.1	47.6	4.2	29.2
9	4/29	62.0	1.53	40.3	+ 1	0.80	61.1	1488	3.0	20.3	3.6	55.0	3.7	25.0
11	5/ 6	62.5	1.57	39.8	± 0	0.83	61.1	1499	3.3	21.9	2.6	40.0	5.7	38.1
12	5/13	65.8	1.56	42.2	+ 5	0.80	64.8	1579	3.0	19.3	4.0	56.2	3.9	24.5
14	5/20	69.5	1.57	44.6	+11	0.77	68.9	1668	2.9	17.2	5.0	66.8	2.7	16.0
17	6/ 5	52.8	1.45	36.4	− 4	0.81	51.8	1267	3.3	26.0	2.5	45.2	3.6	28.4
18	6/11	51.1	1.48	34.5	− 9	0.83	50.0	1226	3.0	24.8	2.2	40.2	4.2	35.0
19	6/17	50.8	1.46	34.8	− 8	0.81	49.9	1219	3.0	24.0	2.5	46.0	3.6	30.0
20	6/24	50.2	1.45	35.3	− 7	0.76	50.2	1204	3.0	24.1	3.7	67.6	1.0	8.3
22	7/ 2	49.4	1.45	34.1	−10	0.77	48.3	1183	3.0	25.2	3.5	66.7	0.9	8.1
23	7/ 8	45.0	1.46	30.8	−19	0.80	44.1	1080	2.8	26.5	2.4	50.3	2.5	23.2
24	7/15	48.1	1.47	32.4	−13	0.81	47.0	1154	3.1	27.0	2.7	53.0	2.3	20.0
26	7/22	51.5	1.48	34.8	− 8	0.80	50.5	1236	3.1	25.3	2.9	51.9	2.8	22.8

[1] Boothby Sandiford standards.

Gaebler ('35) has shown that the growth hormone from the anterior lobe of the pituitary, administered to dogs, produced a marked increase in heat production and a sharp increase in

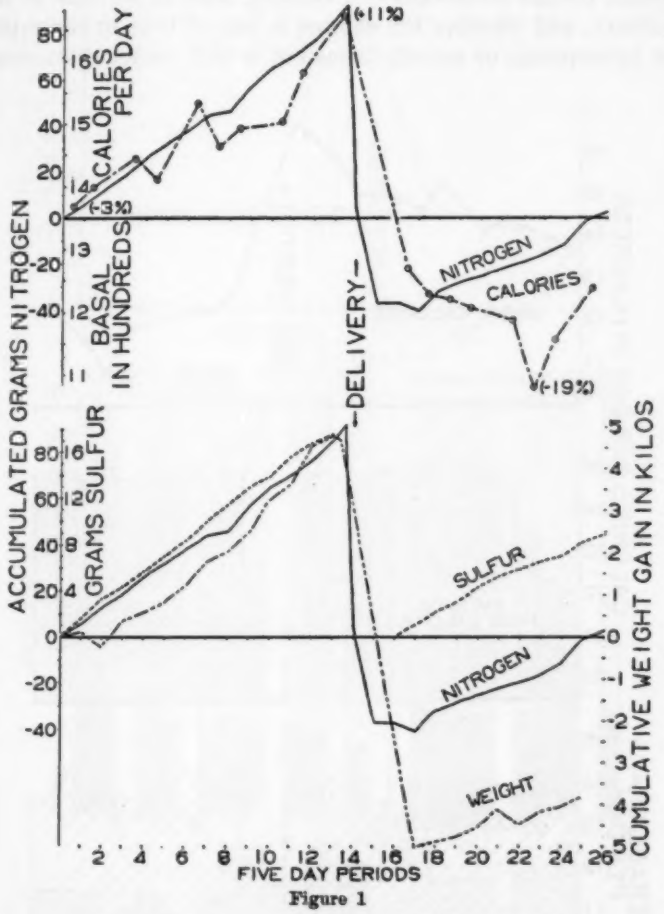

Figure 1

nitrogen retention. Similar changes were noted in thyroparathyroidectomized dogs. Changes simulating these qualitatively, but less marked quantitatively and delayed in their

appearance, were noted with pituitary-like substance from pregnancy urine.

Evidence for a change in the thyroid during pregnancy is definite, though somewhat less striking than in the case of the pituitary, and whether the change is one of true hypertrophy and hyperplasia or merely transient is still under discussion.

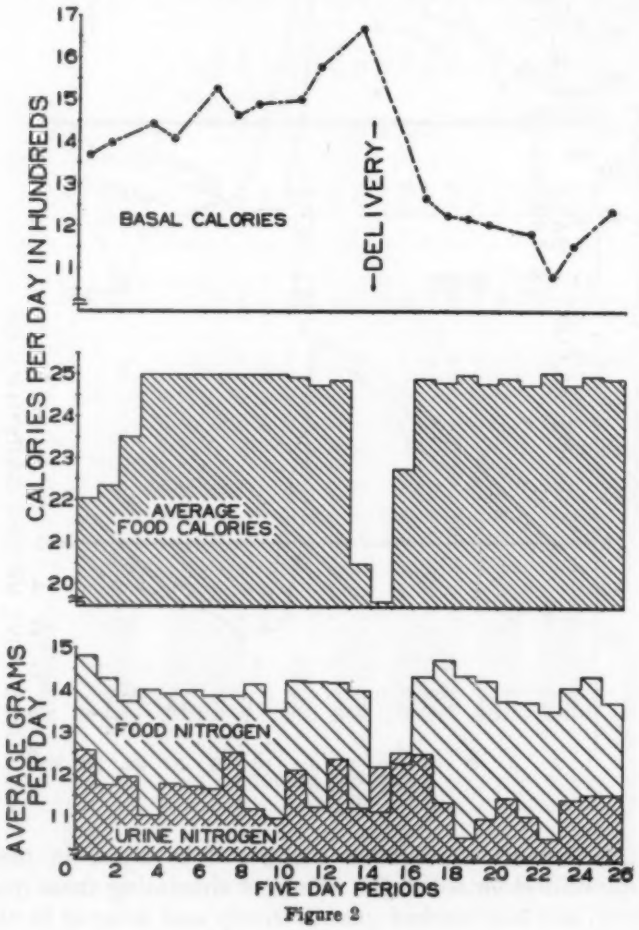

Figure 2

The demonstration of the steady increase in blood iodine from the second to the tenth months and a drop in the first 2 weeks of the puerperium (Borkelmann and Scheringer, '30) together

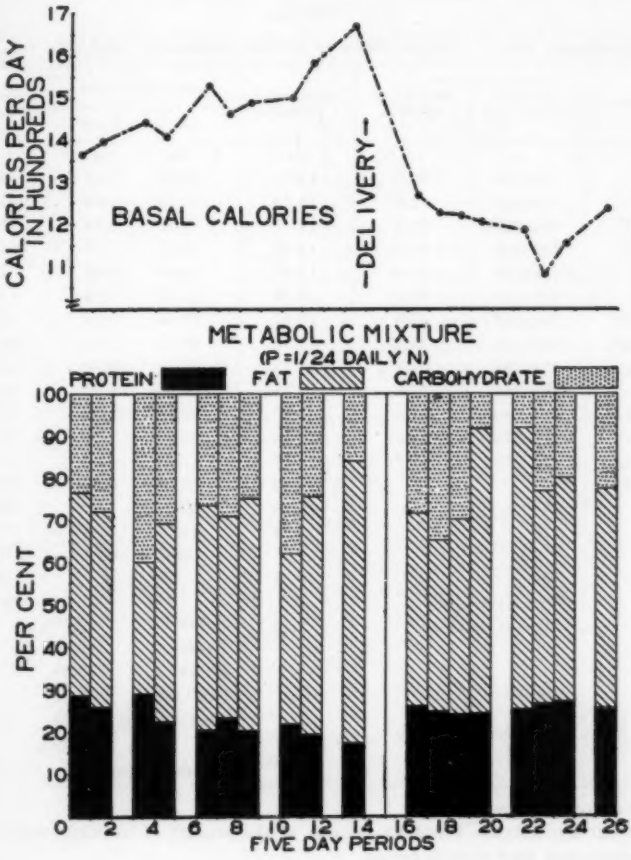

Figure 3

with the demonstration of a glycogen diminishing substance in the blood of pregnant women (Eufinger et al., '29) suggest an increase in thyroid function.

In studying the data here presented the changes noted are very suggestive of those observed by Gaebler. We have calculated what the metabolic mixture would be if the basal hour's

TABLE 2a

Continuous daily[2] *nitrogen balances during pregnancy. Subject—I.M.B.*

PERIOD	DAYS OF PREGNANCY	WEIGHT OF SUBJECT	NITROGEN INTAKE	NITROGEN OUTGO		NITROGEN BALANCE
				Urine	Feces	
		kg.	*gm.*	*gm.*	*gm.*	*gm.*
I	214–218	52.0	14.82	12.46	1.18	+1.18
II	219–223	52.1	14.28	11.72	1.09	+1.47
III	224–229	52.3	13.67	11.92	0.98	+0.77
IV	230–233	52.6	14.02	10.96	1.10	+1.96
V	234–238	52.8	13.96	11.80	0.95	+1.21
VI	239–243	53.0	13.99	11.75	1.04	+1.20
VII	244–248	53.5	13.85	11.62	0.95	+1.28
VIII	249–253	54.1	13.86	12.53	1.01	+0.32
IX	254–258	54.4	14.13	11.21	0.70	+2.22
X	259–263	54.9	13.51	10.95	1.02	+1.54
XI	264–269	55.6	14.20	12.16	1.20	+0.84
XII	270–273	56.0	14.19	11.29	1.15	+1.75
XIII	272–276	56.7	14.17	12.38	1.08	+1.79
XIV	277		14.69	11.05	0.35	+3.29
	278		14.36	10.17	...	+4.19
	279		13.04	11.31	0.84	+0.89
	280[1]		....	8.62	0.84	−10.15
	Mean		13.84			1.21
	Total storage					+83.77[3]
	Day of delivery		9.19	Urine	3.78 gm.	
				Feces	0.84	
				Blood	15.58	
				Placenta	15.48	
				Amniotic fluid	0.35	
					36.03 gm.	
				Balance for day		−26.84 gm.

[1] Vomitus 0.69 gm. N.

[2] Total accumulation found by the sum of the product of the daily storage and the number of days in each period.

[3] Mean of 5-day period.

urine corresponded to one-twenty-fourth of the total urine. This shows that the rising metabolic rate is accompanied by a relative fall in calories derived from protein and a relative

increase from the calories derived from fat. The urine nitrogen is relatively constant showing a very slight tendency to decline.

TABLE 2b

Continuous daily[1] nitrogen balances during puerperium. Subject—I.M.B.

DAY POSTPARTUM	WEIGHT OF SUBJECT	NITROGEN INTAKE	NITROGEN OUTGO					
			Urine	Feces	Breast milk	Blood	Vomitus	Balance
	kg.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	...	11.13	16.95	0.84	...	0.34	...	— 7.00
2	...	11.06	12.08	0.35	...	1.22	...	— 2.59
3	...	12.26	10.15	...	...	1.38	...	+ 0.73
4	...	9.26	7.25	0.34	0.29	0.94	...	+ 0.44
5	...	11.91	16.80	...	0.29	1.34	1.19	— 7.71
6	...	10.21	10.84	0.35	0.29	0.74	1.42	— 3.43
7	...	11.50	13.93	2.17	0.33	0.89	...	— 5.81
8	...	11.39	18.48	1.87	0.33	1.15	...	—10.34
9	...	12.41	7.86	1.25	0.33	0.46	...	+ 2.51
10	...	13.47	12.95	0.75	0.33	0.53	...	— 1.09
11	...	14.72	11.25	0.45	0.33	0.72	...	+ 2.13
Mean		11.76						— 2.92
Total								—32.16
PERIOD	LACTATION							
XVII	12–16	47.0	14.30	12.40	1.43	0.68	0.35	— 0.56
XVIII	17–22	47.3	14.74	11.17	1.67	0.52	0.21	+ 1.17
XIX	23–26	47.5	14.34	10.55	2.14	0.32	0.10	+ 1.23
XX	27–31	47.6	14.22	10.98	1.43	0.98	...	+ 0.83
XXI	32–37	47.6	13.79	11.51	1.53	1.09	...	— 0.34
XXII	38–42	47.6	13.72	11.06	1.23	1.08	...	+ 0.35
XXIII	43–47	47.9	13.51	10.54	1.68	0.50	...	+ 0.79
XXIV	48–52	48.0	14.10	11.52	1.12	0.17	...	+ 1.29
XXV	53–58	48.2	14.36	11.59	1.37	0.03	...	+ 1.37
XXVI	59–62	...	13.73	11.59	1.46	...	0.15	+ 0.53
Mean			14.38					+ 0.67
Total								+33.74

[1] Mean of 5-day period.

It is our feeling that the data here presented favor the view that the rise in the metabolism in pregnancy is to a large extent explained on the basis of an increase in active protoplasmic mass; but that the behavior of the nitrogen excretion and the composition of the metabolic mixture—a relative fall in the protein with a rise in fat—taken in conjunction with

the known facts regarding the changes in the anterior lobe of the pituitary gland, make it seem reasonable to include a hyperactivity of this gland in any explanation that would seem complete.

TABLE 3

Accumulative storage of nitrogen and sulfur with change in body weight during pregnancy, puerperium and lactation

PERIOD	ANTE PARTUM	N	S	CHANGE IN BODY WEIGHT
	days	*gm.*	*gm.*	*gm.*
I	70–66	5.9	1.6	+ 114
II	65–61	13.3	3.3	— 226
III	60–55	17.9	4.3	+ 341
IV	54–51	25.7	5.6	+ 565
V	50–46	31.8	6.9	+ 581
VI	45–41	37.8	8.4	+1200
VII	40–36	44.7	9.9	+1785
VIII	35–31	46.3	11.3	+2019
IX	30–26	57.4	12.9	+2474
X	25–21	65.1	13.9	+3229
XI	20–15	70.1	15.6	+3613
XII	14–11	77.1	16.4	+4547
XIII	10– 6	86.1	17.5	+4786
XIV	5	84.3		+4666
Total accumulation		84.3	17.5	+4666
Loss during delivery		26.8		
Estimated fetal * composition		58.6		
Puerperium 11 days		—32.2		
Total loss		117.6		9677
PERIOD	POST PARTUM	N	S	CHANGE IN BODY WEIGHT
	days	*gm.*	*gm.*	*gm.*
XVII	12–16	—36.1	1.0	—5011
XVIII	17–22	—29.1	2.2	—4884
XIX	23–26	—24.2	3.0	—4768
XX	27–31	—20.0	4.3	—4531
XXI	32–37	—22.1	5.1	—4110
XXII	38–42	—20.3	5.9	—4478
XXIII	43–47	—16.4	6.6	—4180
XXIV	48–52	— 9.9	7.4	—4019
XXV	53–58	— 1.7	8.3	—3809
XXVI	59–62	+ 0.4	8.8	

SUMMARY AND CONCLUSIONS

Data are recorded on the basal metabolism of a young primipara whose mineral balance was determined concurrently. They are interpreted as favoring the view that the changes, while to a large extent understandable as a result of the formation of a new protoplasmic mass, as evidenced by the parallelism between the cumulative nitrogen and sulfur balances and the basal heat production, are at the same time under a hormonal influence contributing to this same synthesis.

ACKNOWLEDGMENTS

We desire to acknowledge gratefully the assistance of the following: Frank Poole, M.D., supervised the collections; Frank Hartman, M.D., and Ruth Spangler, R.N., were responsible for the metabolism determinations; Evelyn Foster, B.S., and Ruth Smithyman, B.S., were in charge of the diets; Mildred MacLaren, R.N., controlled the activity of the patient throughout her stay, and Drs. J. P. Pratt and Kenneth Stolpman supervised the obstetrical care of the patient; O. H. Gaebler, M.D., made many helpful suggestions.

LITERATURE CITED

BORKELMANN, O., AND W. SCHERINGER 1930 Beitrag zur Kenntis der Schilddrusenfunktion und des Jodstoffwechsels in der Gestation. Arch. f. Gynak., Bd. 143, S. 512.

CARPENTER, T. M., AND J. R. MURLIN 1911 The energy metabolism of mother and child just before and just after birth. Arch. Int. Med., vol. 7, p. 184.

EUFINGER, H., H. WIESBADER AND L. FOCSANEANU 1929 Reid Huntsche Reaktion und Schwangerschaft. Arch. f. Gynak., Bd. 136, S. 12.

GAEBLER, O. H. 1935 Effects of thyroparathyroidectomy and carbohydrate intake on action of anterior pituitary extracts. Am. J. Physiol., vol. 110, p. 584.

HARDING, V. J. 1925 Metabolism in pregnancy. Physiol. Rev., vol. 5, p. 279.

HUMMEL, F. C., H. A. HUNSCHER, M. F. BATES, P. BONNER, I. G. MACY AND J. A. JOHNSTON 1937 A consideration of the nutritive state in the metabolism of women during pregnancy. J. Nutrition, vol. 13, p. 263.

KOLDE, W. 1912 Untersuchungen von Hypophysen bei Schwangerschaft und nach Kastration. Arch. f. Gynak., Bd. 98, S. 505.

LUSK, G. 1924 Animal calorimetry; analysis of oxidation of mixtures of carbohydrate and fat. J. Biol. Chem., vol. 59, p. 41.

ROWE, A. W., AND W. C. BOYD 1932 Metabolism in pregnancy; foetal influence on basal rate. J. Nutrition, vol. 5, p. 551.

SANDIFORD, I., AND T. WHEELER 1924 Basal metabolism before, during and after pregnancy. J. Biol. Chem., vol. 62, p. 329.